THE UNIVERSITY OF QUEENSLAND

AUSTRALIA

The Muscle mass, omega-3, exercise, diet and lifestyle study (MODEL):

For women after completion of breast cancer treatment

Cameron McDonald

BSc (Health and Exercise Science) – UNSW

MSc (Nutrition and Dietetics) – Griffith University

A thesis submitted for the degree of Doctor of Philosophy at

The University of Queensland in 2014

School of Human Movement Studies

Abstract

Background

Body weight and composition change after treatment for breast cancer are important considerations when investigating factors affecting risk of disease-free survival. Concurrent loss of lean body mass (LBM) and increase in body fat is common after treatment for breast cancer and are related to the development of metabolic disease. Shorter observational studies have reported significant associations between body composition changes, inflammation, cardiac death and increased risk of metabolic syndrome. Thus, studies that aim to increase the understanding of how body composition change affects outcomes in this population are required. Numerous studies have investigated the mechanisms and quantity of body fat change, however, quantity and causes of LBM loss after treatment are not fully understood.

Exercise interventions have been shown to improve body composition (LBM and body fat%), waist girth, aerobic fitness and other risk factors for metabolic syndrome without reducing body weight. Nutrition interventions have been shown to reduce body weight and body fat% that is accompanied with an improvement in metabolic health. However, the reduction in weight includes potentially detrimental reductions in LBM and significantly increased risk of sarcopenia. The combination of nutrition plus exercise seems to maintain LBM and elicit concurrent body fat and/or body weight reductions. Long chain omega-3 fatty acids (LCn-3) have been associated with improved cardio-metabolic health, have a theoretical yet inconsistent effect on adiposity, are associated with decreased inflammation, and more recently have been shown to improve the response of LBM to an anabolic stimulus. Thus, they present as a relevant clinical option for this population, yet no evidence currently exists after treatment for breast cancer.

Therefore, this PhD research had two aims: the primary aim was to examine the independent and combined effects of an exercise and nutrition program and LCn-3 supplementation on LBM change, QOL and chronic inflammation soon after completion of treatment for breast cancer. This was done by conducting a 6-month 3-arm randomised controlled trial that compared three conditions: 1) LCn-3 supplementation only (N-3); 2) LCn-3 supplementation plus a 12-week group exercise and nutrition lifestyle program (Ex+N-3); and, 3) the lifestyle program plus placebo - olive oil (Ex+OO). The secondary aim was to explore baseline cross-sectional associations between body composition (in particular LBM) and LCn-3 intake, treatment, demographical and lifestyle factors after completion of treatment for breast cancer. For the scope of this PhD thesis, the investigation targeted women who had completed treatment of breast cancer within the last 12 months, and were considered ‘disease free’ at entry to the trial. The thesis is presented as both unpublished work, and a series of published and submitted manuscripts.

Due to slower than expected recruitment, 49 participants were included in the trial, and were generally representative of women who have been diagnosed with breast cancer in Australia.
In the six month randomised controlled trial, all three groups experienced maintenance of LBM, with no significant differences between groups after 24 weeks. Compared to women who consumed LCn-3 supplements or participated in the lifestyle program separately, those exposed to both interventions were more likely to experience a greater amount of body weight and waist and hip girth reduction. Quality of life (QOL) improved for all groups, while C-reactive protein (CRP) levels did not change throughout the intervention. Secondary analyses indicated that LCn-3 supplementation was associated with improved physical function and maintenance of grip strength independent of exercise and nutrition. Limitations of the intervention were lower than expected recruitment rate, and the effectiveness of the resistance training program may have been reduced as a result of the use of elastic resistance equipment.
In terms of the secondary aim (cross-section at baseline), after adjusting for weight and age, the major determinants of LBM after treatment were higher levels of aerobic fitness and the ability to perform a greater number of push ups. Erythrocyte levels of LCn-3, energy and protein intake, CRP and treatment related variables were not associated with body composition after treatment for breast cancer.
This thesis provides new insight into the synergy of LCn-3 and an exercise and nutrition lifestyle program in a population of women who have been treated for breast cancer. Combining LCn-3 supplementation with best practice nutrition and exercise advice is a consideration for clinicians aiming to prevent and improve adverse body composition change after treatment. Longer-term research investigating the preventive effect of LCn-3 and exercise on development of metabolic syndrome and breast cancer related morbidity and mortality should be undertaken. Finally, our novel findings indicate that muscle function is strongly associated with weight adjusted LBM after treatment, and its use as a measure of health warrants further investigation in determining the overall health of breast cancer survivors.

Declaration by author

This thesis *is composed of my original work, and contains* no material previously published or written by another person except where due reference has been made in the text. I have clearly stated the contribution by others to jointly authored works that I have included in my thesis.

I have clearly stated the contribution of others to my thesis as a whole, including statistical assistance, survey design, data analysis, significant technical procedures, professional editorial advice, and any other original research work used or reported in my thesis. The content of my thesis is the result of work I have carried out since the commencement of my research higher degree candidature and does not include a substantial part of work that has been submitted *to qualify for the award of any* other degree or diploma in any university or other tertiary institution. I have clearly stated which parts of my thesis, if any, have been submitted to qualify for another award.

I acknowledge that an electronic copy of my thesis must be lodged with the University Library and, subject to the General Award Rules of The University of Queensland, immediately made available for research and study in accordance with the *Copyright Act 1968*.

I acknowledge that copyright of all material contained in my thesis resides with the copyright holder(s) of that material. Where appropriate I have obtained copyright permission from the copyright holder to reproduce material in this thesis.

Cameron K McDonald

Publications during candidature

Publications Under Review

1. The Muscle mass, Omega-3, Diet, Exercise and Lifestyle (MODEL) Study – a randomised controlled trial in women who have completed treatment for breast cancer – Submitted for review – Journal of Cachexia, Sarcopenia and Muscle

Manuscripts Published in Peer-Reviewed Journals

1. McDonald C, Bauer J, Capra S, Waterhouse M (2013) Muscle function and omega-3 fatty acids in the prediction of lean body mass after breast cancer treatment. *SpringerPlus* (2013), 2: 681 doi: 10.1186/2193-1801-2-681.

2. McDonald, CK., Bauer, J., Capra, S., Cole, J. The Muscle mass, Omega-3, Diet, Exercise and Lifestyle (MODEL) study protocol – a randomised controlled trial for women who have completed breast cancer treatment. *BMC Cancer* (2014) 14 (1): 264. Journal Impact Factor: 3.33

3. McDonald, CK., Bauer, J., Capra, S., Cole, J. Omega-3 fatty acids and changes in LBM-alone or in synergy for better muscle health? Canadian Journal of Physiology & Pharmacology. 91: 459–468 (2013) dx.doi.org/10.1139/cjpp-2012-0304. Journal Impact Factor: 1.56

4. McDonald, CK., Bauer, J., Capra, S. Body composition and breast cancer – a role for lean body mass. *Cancer Forum* (2011) 35 (2). Journal impact factor: NA

Peer Reviewed Conference Proceedings

McDonald, CK (2011) Lean body mass, breast cancer and metabolic syndrome: A role for nutrients. *Universitas 21 Graduate Research Conference on Food Proceedings, Semenyih, Malaysia*

McDonald, CK (2011) Active nutrients: Management of cancer-related myopenia through exercise and nutrition. *Australian Lifestyle Medicine Association Annual Conference, Manly, Australia*. Presented findings from published manuscript 1

McDonald, CK (2012) An update on Nutrients & Exercise for Body composition - Feel the Synergy. *Australian Lifestyle Medicine Association Annual Conference, Manly, Australia.* Presented findings from published manuscript 2

Publications included in this thesis

Publications included in Chapter 2 – Literature Review

1. McDonald, Bauer, Capra (2011) Body composition and breast cancer – a role for lean body mass. Cancer Forum 35 (2) - **Published**

Contributor	Statement of contribution
McDonald, C (Candidate)	Contributed to the concept and design of the review topic (80%) Literature Search and Review (100%) Drafting of the manuscript (100%)
Bauer, J	Contributed to the concept and design of the review topic (15%) Review and Critical Appraisal of manuscript (50%)
Capra, S	Contributed to the concept and design of the review topic (5%) Review and Critical Appraisal of manuscript (50%)

2. McDonald, Bauer, Capra, Cole (2012) Omega-3 fatty acids and changes in LBM-alone or in synergy for better muscle health? Can. J. Physiol. Pharmacol. 91: 459–468 (2013) dx.doi.org/10.1139/cjpp-2012-0304 - **Published**

Contributor	Statement of contribution
McDonald, C (Candidate)	Contributed to the concept and design of the review topic (90%) Literature Search and Review (100%) Drafting of the manuscript (100%)
Bauer, J	Contributed to the concept and design of the review topic (5%) Review and Critical Appraisal of manuscript (50%)
Capra, S	Contributed to the concept and design of the review topic (5%) Review and Critical Appraisal of manuscript (50%)

Publications included in Chapter 3 -Methods

3. McDonald, CK., Bauer, J., Capra, S., Cole, J. The Muscle mass, Omega-3, Diet, Exercise and Lifestyle (MODEL) study protocol – a randomised controlled trial for women who have completed breast cancer treatment. *BMC Cancer* (2014) 14 (1): 264. **Published**

Contributor	Statement of contribution
McDonald, C (Candidate)	Contributed to the concept and design of the methods (60%) Selection and justification of measures used (80%) Drafting of the manuscript (100%) Statistical analysis design: Power calculation and primary analysis (20%)
Bauer, J	Contributed to the concept and design of the review topic (20%) Review and Critical Appraisal of manuscript (50%) Selection and justification of measures used (10%) Statistical analysis design: Power calculation and primary analysis (10%)
Capra, S	Contributed to the concept and design of the review topic (20%) Review and Critical Appraisal of manuscript (50%) Selection and justification of measures used (10%) Statistical analysis design: Power calculation and primary analysis (10%)
Cole, J	Statistical analysis design: Power calculation and primary analysis (60%)

Publications included in Chapter 4 - Results

4. McDonald C, Bauer J, Capra S, Waterhouse M (2013) Muscle function and omega-3 fatty acids in the prediction of lean body mass after breast cancer treatment. *SpringerPlus* (2013), 2: 681 doi: 10.1186/2193-1801-2-681. **Published**

Contributor	Statement of contribution
McDonald, C (Candidate)	Contributed to the concept and design of the study (70%) Data collection (100% - 10% by research assistant) Data entry (100%) Data analysis (50%) Drafting of the manuscript (100%) Reviewed and edited the manuscript (30%)
Bauer, J	Contributed to the concept and design of the study (20%) Reviewed and edited the manuscript (50%)
Capra, S	Contributed to the concept and design of the study (10%) Reviewed and edited the manuscript (20%)
Waterhouse, M	Statistical analysis (50%)

Publications included in Chapter 5 - Results

5. The Muscle mass, Omega-3, Diet, Exercise and Lifestyle (MODEL) Study – a randomised controlled trial in women who have completed treatment for breast cancer – **Submitted for review** Journal of Cachexia, Sarcopenia and Muscle

Contributor	Statement of contribution
McDonald, C (Candidate)	Contributed to the concept and design of the study (70%) Data collection (100% - 10% by research assistant - acknowledged) Data entry (100%) Data analysis (70%) Drafting of the manuscript (100%) Reviewed and edited the manuscript (30%)
Bauer, J	Contributed to the concept and design of the study (20%) Reviewed and edited the manuscript (50%)
Capra, S	Contributed to the concept and design of the study (10%) Reviewed and edited the manuscript (20%)
Duncan, E	Statistical analysis (30%)

Contributions by others to the thesis

Professor David Jenkins contributed to exercise prescription considerations and program design. Yun-Chi Hung was research assistant and responsible for all body composition analyses and Lymphoedema index analysis. Dr Michael Leveritt aided in structure and gave instrumental feedback for published manuscript 2.

Statement of parts of the thesis submitted to qualify for the award of another degree

None

Acknowledgements

There are many colleagues and friends who have made this journey achievable. Firstly, I need to thank A/Prof Judy Bauer and Prof Sandra Capra. Not only did they plant the seed of this trial before I was aware of it, but they were extremely patient with me as I attempted to get my head around the requirements of completing this project. Both have been excellent sounding boards for ideas for the intervention, and both have been instrumental in my learning of getting a project to a finished product.

Dr Michelle Palmer and Prof David Jenkins, as part of my review team were helpful in their honest guidance of the project and always made themselves available when asked, many thanks to them.

Deb Noon needs to be mentioned! As the co-ordinator of the post-grad herd, there was never a time when the simplest and most trivial of administrational questions was too mundane. Moving through the School of Human Movements was made to be seamless and fun with Deb as coordinator, and her ongoing support and encouragement always made the days at UQ a little brighter.

The funding I received from the Wesley Research Institute was the only reason the study had a life initially. The staff at the WRI and the use of their facilities made the whole process of running the intervention delightful.

As a result of this funding and publicity, it was a true delight to receive a fantastic donation from the 'Pink Accessories' group at Clayfield College. It was an honour to have our study chosen as the recipient of the funds raised as a result of a wonderful Breast Cancer charity day at the College, and I feel that the young women in charge of this project have many amazing years ahead.

Finally in terms of financial support, I was very thankful for the support of the APA scholarship program. Without this program, performing this project would not have been possible.

During my PhD I was able to experience 3 months abroad in Denmark, working on similar projects and completing write up of the thesis. This would not have been possible without the help of a GSITA grant that supported part of my travels over there.

Personally, there are a huge number of friends that have made the last 3.5 years more joyous, too many to mention. However, it's extremely important for me to thank my family who have always allowed me to follow my own path.

Keywords

Breast cancer, lean body mass, body composition, exercise, omega-3, nutrition, intervention, randomised controlled trial

Australian and New Zealand Standard Research Classifications (ANZSRC)

ANZSRC code: 111199, Nutrition and Dietetics, Not elsewhere classified, 70%

ANZSRC code: 110602, Human movement and Sports Sciences, Exercise Physiology, 30%

Fields of Research

FoR Code: 1111 Nutrition and Dietetics, 70%

FoR Code: 1106 Human movement and Sports Science, 30%

Contents

List of Tables

List of Figures

List of Abbreviations

%time>moderate	% of the time spent in more than or equal to moderate intensity activity measured by accelerometry
1-RM	1 repetition maximum
AA	Arachadonic acid
ACSM	American College of Sports Medicine
ADP	Air Displacement Plethysmography
AEP	Accredited Exercise Physiologist
AFFQ	Arizona Food Frequency Questionnaire
AI(s)	Aromatase Inhibitor(s)
ALBM	Appendicular lean body mass
APD	Accredited Practising Dietitian
BGL	Blood glucose levels
BIA	Bio electrical impedance
BMD	Bone mineral density
BMI	Body mass index
BrCa	Breast Cancer
CHO	Carbohydrates
CON	Control group
CRP	C-Reactive protein
CT-scan	Computed Topography-scan
CTx	Chemotherapy
CV	Cardiovascular
CVD	Cardiovascular Disease
DEXA	Dual-Energy X-ray Absorptiometry
DHA	Docosahexanoic acid
DHQ	Dietary Habits Questionnaire
DPA	Docosapentanoic acid
Dx	Diagnosis
EP+N-3	Group name: Exercise and Nutrition Lifestyle program and LCn-3 supplementation
EP+OO	Group name: Exercise and Nutrition lifestyle program plus placebo capsules (olive oil)'
EPA	Eicosapentanoic acid
ESSA	Exercise and Sports Science Australia

FACT-An	Functional Assessment for Cancer Therapy - Anaemia scale
FACT-B+4	Functional Assessment of Cancer Therapy - Breast cancer + 4 items
FACT-F	FACT - Fatigue scale
FACT-G	FACT - General
FBI	Fat Booters Incorporated
g	gram
GCS	Greene Climacteric Scale
HAQ-DI	Health Assessment Questionnaire - Disease index
HDL	High density lipoprotein
HOMA	Homeostasis Model Assessment
HR	Heart Rate
HRs	Hazard Ratios
HREC	Human Research Ethics Committee
Ht	Height
INT	Intervention group
ITT	Intention to treat
kg	kilogram
kJ	kilojoule
L-DEX	Lymphoedema index
LBM	Lean body mass
LCn-3	Long chain omega-3 fatty acids
LDL	Low density lipoprotein
m	metre
MPS	Muscle protein synthesis
MRI	Magnetic Resonance Imaging
OW	Overweight
N-3	The group receiving LCn-3s only
PA	Physical Activity
PAQ	Physical activity questionnaire
PAR-Q	Physical Activity Readiness Questionnaire
PBOO	Plant based olive oil diet plan
Postmeno	Postmenopausal
QOL	Quality of life

RBC	Red blood cell/erthrocyte
RER	Respiratory exchange ratio
RMANOVA	Repeated Measures Analysis of Variance
RPE	Relative Perceived Exertion
RTx	Radiotherapy
Rx	Treatment
SES	Socioeconomnic Status
StageTM	Stage completed on the treadmill sub-maximal Vo2 test
T2DM	Type 2 Diabetes Mellitus
Tchol	Total Cholesterol
TP1	Time point 1
TP2	Time point 2
TP3	Time point 3
UCH	Uniting Care Health
WHR	Waist-to-hip ratio
wk	Week
Wt	Body weight (kg)

Chapter 1 – Introduction

Breast cancer is the most common cancer diagnosis for women, with it's global incidence totaling 1.38 million in 2008 (Ferlay et al. 2010). In Australia alone, annual incidence has increased from 5310 to 13 567 over the last 2.5 decades (Australian Institute of Health and Welfare & Cancer Australia 2012). Fortunately, due to improved diagnostic, surgical and adjuvant (chemotherapy and radiotherapy) treatments for breast cancer, 5-yr survival rates have increased to 89.3% (Australian Institute of Health and Welfare & Cancer Australia 2012). However, all women with breast cancer are at equal, if not greater, risk of dying from heart disease as they are from breast cancer itself (Hanrahan et al. 2007), and treatment may be a factor that elevates this cardiovascular disease (CVD) risk (Rock and Demark-Wahnefried 2002).

After treatment for breast cancer, presence of higher BMI, abdominal obesity, increased inflammation and/or hyperglycaemia are strongly linked to an increased risk of mortality and recurrence (Protani et al 2010, Pierce, Ballard-Barbash et al 2009) and metabolic disease (Healy et al. 2010). However, after treatment total body weight gains commonly range up to 5kg in the one to three years following treatment (Demark-Wahnefried, Campbell, and Hayes 2012), with the greatest rate of change occurring within the first six to 12 months following treatment (Makari-Judson et al 2007). A combination of epidemiological and intervention studies have indicated that, compared to body weight increase or substantial decrease (>3kg change), weight stability (Nichols et al 2009, Caan et al 2008) or moderate body weight loss (<3kg change) (Ligibel 2012, Chlebowski et al 2006) are related to improved survival. Currently for early stage breast cancer, a vast majority of women are more likely to gain total body weight than to experience weight loss after treatment (Demark-Wahnefried et al 2012). However unlike weight gain in healthy populations that typically consist of lean and fat mass increases, breast cancer survivors commonly experience loss of lean body mass (LBM) and concurrent increases in visceral (Cheney, Mahloch, and Freeny 1994) and general fat mass (Rooney and Wald 2007, Harvie 2010).

Increases in adipose tissue have been shown to increase levels of C-reactive protein (CRP) and serum amyloid A (SAA) (Dee et al. 2012), which have in turn been associated with increased risk of all cause and cardiovascular (CVD) mortality in breast cancer survivors (Pierce, Ballard-Barbash, et al. 2009). A limited number of studies have partly explained mechanisms underpinning loss of LBM after treatment for breast cancer. However, chemotherapy (Sheean, Hoskins, and Stolley 2012), a reduction in physical inactivity (Irwin et al. 2005), and chronic inflammation (Mourtzakis and Bedbrook 2009) have been proposed as potential causes. Also, intentional weight

loss through dietary energy restriction may promote a greater rate of LBM loss. In contrast, treatment with aromatase inhibitors may positively influence LBM (van Londen et al. 2011). Overall, there is limited research describing modifiable risk factors for LBM and general body composition change in this population.

Physical activity is an important consideration when assessing changes in general health and LBM after a diagnosis of breast cancer. A growing evidence base indicates that contraction of skeletal LBM through exercise has numerous positive effects on immune and hormonal function (Pedersen and Febbraio 2012). Observational data indicates that increased levels of physical activity (brisk walking 3 hours/day) have been associated with improved mortality and reduced recurrence after a diagnosis of breast cancer (Ibrahim and Al-Homaidh 2010). While physical inactivity is known to contribute to systemic pathophysiological changes such as insulin resistance, cardiovascular disease, colon cancer and osteoporosis (Hamilton, Hamilton, and Zderic 2007). Thus, considering the greater risk of mortality and metabolic syndrome in this population, assessment of physical activity and inactivity is important for ongoing morbidity and mortality.

Physical activity interventions have been shown to be effective in maintaining volume and function of LBM in breast cancer survivors (Schmitz et al. 2010). In addition, formal physical activity after breast cancer treatment has been shown to reduce risk factors for metabolic syndrome such as aerobic capacity (Courneya et al 2007), waist girth (Guinan et al 2013; Fernandez et al, 2013), and improve both overall, breast, psychosocial and physical function subscales of quality of life (QOL) following treatment (Herrero et al, 2006; Fernandez-Lao et al, 2013; Courneya et al, 2007; Matthews et al, 2007; Ohira et al, 2006). Therefore, prescribed exercise may be important in improving not only survival and morbidity, but enhancing QOL and body composition as well.

Dietary interventions in women who have completed treatment for breast cancer have shown that moderate weight loss through a low fat diet (2.7kg) may improve survival over 4 years (Chlebowski et al 2006). A shorter study (<12 months in length) has shown that greater weight loss (>5kg) may improve risk factors for metabolic disease (Thomson et al 2010), yet this occurred at the expense of an increased incidence of sarcopenia. Thus, if future trials are to explore the potential metabolic benefits of body weight loss in this population, strategies to ameliorate LBM loss are important considerations.

In both healthy and cancer populations, resistance exercise training (RET) is associated with increased LBM volume and function (Schmitz et al 2005, Courneya et al 2007), while some

evidence indicates aerobic exercise may also prevent loss of LBM in breast cancer survivors (Irwin et al 2009). Furthermore, specific nutrients such as LCn-3 (McDonald, Bauer, and Capra 2013) and branched chain amino acids (BCAAs)(Breen and Phillips 2013) have been shown to provide additional benefit to LBM accretion alone or in combination with exercise training. However, neither LCn-3s nor BCAAs have been investigated alone or in conjunction with exercise training in a population of women who have completed treatment for breast cancer.

LCn-3s have a well-established role in decreasing inflammation in healthy (Calder 2012) and cancer populations (Murphy et al 2012). There is growing evidence that indicates improved breast cancer related outcomes after higher intake of LCn-3 (Patterson et al 2013, de Lorgeril 2014). In addition, they may also enhance body composition improvements through improvements in adiposity (Kabir et al, 2007; Hill et al, 2007), LBM accretion (Smith et al, 2011) and functional change (Rodacki et al, 2012). Furthermore, LCn-3 supplements are readily available, safe and widely consumed, thus they are an excellent candidate for further investigation in this population.

Therefore, the primary aim of this thesis was to examine the independent and combined effects of an exercise and nutrition program and LCn-3 supplementation on LBM change, QOL and chronic inflammation up to 12 months after completion of treatment for breast cancer.
The secondary aim was to explore associations between body composition (in particular LBM) and LCn-3 intake, treatment, demographical and lifestyle factors after completion of treatment for breast cancer. .

Overview of the thesis

Chapter 2

Review of the literature inclusive of two review publications:

The unpublished literature review includes:

- A detailed summary of the current literature surrounding body composition changes and their sequelae after treatment for breast cancer.
- A comprehensive literature review of exercise interventions, dietary interventions and combined exercise dietary interventions and their effect on body composition change.

Published reviews:

1. McDonald, Bauer, Capra (2011) Body composition and breast cancer – a role for lean body mass. Cancer Forum 35 (2).

This manuscript explains the importance of lean body mass for those at risk and diagnosed with breast cancer, and briefly outlines the current literature and potential interventions to reduce or prevent adverse LBM changes after treatment.

2. McDonald, Bauer, Capra, Cole (2012) Omega-3 fatty acids and changes in LBM-alone or in synergy for better muscle health? Can. J. Physiol. Pharmacol. 91: 459–468 (2013) dx.doi.org/10.1139/cjpp-2012-0304

This manuscript addresses the current literature for the effects of LCn-3 on LBM change, and how these effects may be augmented through addition of concurrent anabolic interventions. In addition, the review aimed to determine the appropriate dosage of LCn-3 required to effectively test its efficacy in LBM change

Chapter 3 - Methods

Methods – inclusive of one published manuscript 3, and an extended description of additional measures not included in the publication.

McDonald, CK., Bauer, J., Capra, S., Cole, J. The Muscle mass, Omega-3, Diet, Exercise and Lifestyle (MODEL) study protocol – a randomised controlled trial for women who have completed breast cancer treatment. *BMC Cancer* (2014) 14 (1): 264. Journal Impact Factor: 3.33

Chapter 4 – Baseline Results

Cross sectional results from baseline assessment – inclusive of one published manuscript. In addition, extended unpublished results and their respective discussions are included after the paper to provide results for measures not included the published manuscript.

McDonald C, Bauer J, Capra S, Waterhouse M (2013) Muscle function and omega-3 fatty acids in the prediction of lean body mass after breast cancer treatment. *SpringerPlus* (2013), 2: 681 doi: 10.1186/2193-1801-2-681.

This cross-sectional analysis of the data set at baseline addresses the secondary aim of the thesis. There are currently no studies that have linked muscle strength or function to body composition outcomes in breast cancer survivors. This paper contributes novel evidence in this area.

Chapter 5 – Intervention Results

Reporting of results from the randomised controlled trial. This chapter includes one manuscript under review, and results and discussion from exploratory analyses that were not included in the manuscript.

The Muscle mass, Omega-3, Diet, Exercise and Lifestyle (MODEL) Study – a randomised controlled trial in women who have completed treatment for breast cancer – Submitted for review – *Breast Cancer Research & Treatment*

This paper addresses the primary aim of the thesis.

Chapter 6 – Discussion and Conclusions

Discussion, Limitations and strengths, Conclusions and Future Directions

Chapter 2 - Literature review

2.0 Overview

This chapter begins with an unpublished review of the literature that describes the influence of body composition on breast cancer risk, and prognosis following treatment. The focus of this chapter is to elucidate the following: what is considered to be the recommended body composition outcome for breast cancer survivors when considering overall mortality and morbidity. The second part of this chapter is a published review that summarises these findings and collates information from non-breast cancer populations to suggest potential nutritional aids that could be utilized to better maintain LBM after treatment for breast cancer.

Following this the second published manuscript describes the considerations for use of LCn-3 as an adjunct to exercise and nutrition interventions. The paper describes detail on appropriate dosing, and the need for LCn-3 to be combined with an anabolic stimulus in order to have an effect on LBM.

2.0.1 Literature search method

A literature search was carried out using MEDLINE and Pubmed databases. Selected studies and review articles were hand-searched for additional relevant references, with forward citation searching completed through Google Scholar and within journal databases.

Quality assessment

Studies were assessed for quality (relevance and validity) according to the guidelines set out in the Evidence Analysis Manual published by the Academy of Nutrition and Dietetics, 2010 (Academy of Nutrition and Dietetics 2010). High quality studies are assigned a (+); Studies of neutral quality are assigned (0); studies of poor quality were assigned a (-).

In addition, articles were assigned a level of evidence according to the National Health & Medical Research Council (NH&MRC) evidence based clinical practice guidelines.

ADA and NH&MRC guidelines can be found in Appendix 5. Full details of the search terms used in the literature review can be found in Appendix 5.

Quality of Body Composition Assessment Tools

Various instruments for measurement of body composition are reported in the literature. Within the literature review, the term ‘high quality measure’ is used when referring to the use of dual energy x-ray absorptiometry (DEXA), air displacement plethysmography (ADP), magnetic resonance imagery (MRI), computed-tomography scans (CT scans) and under-water-weighing (UWW) to measures body composition. For the purposes of this review, lower quality measures in determining

approximate fat and muscle content of the body are considered to be skinfold assessment, bio-electrical impedance, and simple girth (waist and hip) measures. While we consider that 'high quality' instruments produce more reliable, all results generated from body composition analysis will have certain error due to indirect measurement and assumptions made.

Recent analyses and investigation into reliability of DEXA indicate that results can vary considerably depending on the technicians' and participants' preparation and performance during the measurement. Considerations such as food and fluid consumption before the test, and specific limb position/rotation can create significant variance in serial measures (Nana et al 2014). All of the studies in the review below were performed before 2014, and as a result have not consistently reported or considered these factors, which decreases the reliability of results reported (Nana et al 2014). Similarly, ADP and UWW use the assumption of thoracic volume in the calculation of its body composition result. Due to biological variation this will contribute to inaccuracies in one-off measures (Fields et al 2002). However considering the small changes in lung volume over one to six months, this may not affect the reliability of serial measures. In contrast, length of hair and volume of the hair under the cap can influence the total body volume particularly for ADP (Fields et al 2002). Reliability of MRI and CT scans is related to technical precision, i.e. how effectively muscle and fat is demarcated by the technician. Weaknesses of MRI and CT scans in determining body composition are related to their estimate of volume of fat tissue, and their inability to separate adipose tissue and the actual fat within the tissue. However, MRI is an excellent instrument to measure abdominal adipose tissue (Wells et al 2006).

Granted the presence of these known errors, these measures are still considered to be more accurate and reliable in determining muscle and fat compartments than skinfold measures and bio-electrical impedance, thus have been considered as higher quality (Wells et al 2006).

2.1 Body composition and breast cancer: risk, prognosis and survival

Understanding the effects of body composition change on breast cancer and overall health outcomes is important to the development of a safe treatment protocol. This review aims to discuss what is currently considered the optimal body composition before and after treatment for breast cancer.

2.1.1 Body mass and composition and breast cancer risk

BMI and breast cancer risk

Collectively, studies investigating the association between body composition and risk of breast cancer have only used total body weight/body mass index (BMI), waist and hip girth, and/or waist-

to-hip ratio as the measures of body size. Changes in these measures have been associated with risk of breast cancer, however the literature indicates differences in relationships for pre- and post-menopausal women.

A meta-analysis of 345 studies with a total of 31039 women indicated that for European and North American women, each 5kg/m^2 increase in BMI was related to a decreased risk of premenopausal breast cancer (Hazard ratios (HRs) 0.92; 95% CI: 0.88, 0.97), while the same increment in change was related to a higher risk of post-menopausal breast cancer (HRs 1.12; 95%CI 1.08, 1.16) (Renehan et al. 2008). On the other hand, a higher BMI was correlated to an increased risk of breast cancer in Asian-Pacific populations for both pre- and postmenopausal women (Renehan et al. 2008).

Waist and Waist-to-hip ratio

Menopausal status also seems to influence the effect of waist and waist-to-hip ratio (WHR) on breast cancer risk. A systematic review (Harvie, Hooper, and Howell 2003) and one meta-analysis (Connolly et al. 2002) both reported that compared to those in the lowest quartiles of waist girth (Harvie, Hooper, and Howell 2003) and waist-to-hip ratio (WHR) (Harvie, Hooper, and Howell 2003, Connolly et al. 2002), respectively, those in the highest quartile experienced the greatest risk of pre- and postmenopausal breast cancer risk. After controlling for BMI, Connolly et al (2002) reported that the relationship was maintained in postmenopausal women, however Harvie et al (Harvie, Hooper, and Howell 2003) found that the association disappeared in premenopausal women after controlling for BMI. Thus, it is this author's (the candidate's) suggestion that abdominal obesity measured by waist girth or WHR, but not BMI, may be related to premenopausal breast cancer, while general obesity may be the more powerful measure of body composition in predicting postmenopausal breast cancer risk.

Lean body mass and body fat%

Two prospective cohort studies (Mellemkjaer et al. 2006, MacInnis et al. 2004), one repeated measure time series study (Lahmann et al. 2003), and one case control study(Ronco et al. 2009) have evaluated the effect of body fat% and LBM on breast cancer incidence. The results of the first three trials were limited to postmenopausal women measured with BIA. In contrast, Ronco et al (Ronco et al. 2009) included women in pre- and postmenopausal states and used skinfold plus additional anthropometric measures such as, femoral condyle and bistiloid diameters for body composition assessment.

Two of four studies reported that a higher LBM is positively associated with incidence of breast cancer (Mellemkjaer et al. 2006, MacInnis et al. 2004), while three of four studies have indicated an

increased risk for those in the highest quartile for body fat% (MacInnis et al. 2004, Lahmann et al. 2003, Ronco et al. 2009). However, it is possible the association of LBM and increased risk of breast cancer may not be as strong as indicated in the 2 studies finding a positive result. A higher total LBM will typically be found secondary to both higher body weight and BMI, as 20-40% of normal weight gains are attributed to LBM increases (Forbes et al. 1996). Therefore, women with a higher BMI are more likely to also have a higher absolute lean mass along with higher absolute fat tissue. Therefore the increased risk may be related to a higher BMI than measure of LBM. Supporting this, Ronco et al (2009) reported that less appendicular LBM, and a greater fat-to-muscle were associated with an increased risk of breast cancer (Ronco et al. 2009). However, the study was conducted in a Uruguayan population of very low socioeconomic status. Furthermore the body composition calculation used had not been validated in published literature, thus it's generalisability to Western populations may be limited (Ronco et al. 2009). However, relationships between muscle and fat, rather than individual measures of either compartment warrant further investigation.

Taking these data together, a higher total body weight, which is likely to result in higher waist girth, body fat% and absolute LBM are likely to increase the risk of breast cancer in western postmenopausal populations. However, lower total body weight and higher abdominal obesity are related to higher incidence of breast cancer in women who are premenopause. Measures of body composition that include a measure of body weight adjusted LBM may reveal different relationships, however confirmation studies are needed.

2.1.2 Body composition at breast cancer diagnosis

Higher BMI after a diagnosis of breast cancer is related to an increased risk of mortality and recurrence regardless of menopausal status. Two recent meta-analyses of epidemiological studies indicated that compared to women who were non-obese, women who were obese at the time of diagnosis had a 25(Niraula et al. 2012) to 33%(Protani, Coory, and Martin 2010) increased risk of overall mortality. Similar results were noted for breast cancer specific mortality (Protani, Coory, and Martin 2010, Niraula et al. 2012). These results were unchanged when women were stratified by menopausal status (Niraula et al. 2012), oestrogen receptor status (Niraula et al. 2012, Protani, Coory, and Martin 2010), HER-2 receptor status(Protani, Coory, and Martin 2010), or if WHR was used as the measure of body composition (Protani, Coory, and Martin 2010). In terms of breast cancer recurrence, a 10-year follow up of a large Danish cohort (N=18 967) indicated the risk of developing distant metastases was 46% greater for obese (BMI >30kg.m^{-2}) versus non-obese women (Ewertz et al. 2011). Of note, both chemotherapy and endocrine therapy seemed to be less effective after 10-years in obese women (Ewertz et al. 2011).

Potential reasons for the relationship of a higher BMI to poorer prognosis have been proposed but not yet rigorously tested. Niraula et al reported that compared to women of normal weight, women who are obese tend to present with larger tumours, have more advanced disease, may experience under-dosing of chemotherapy or enhanced toxicity which reduces compliance (Niraula et al. 2012). Direct mechanisms for breast cancer development include obesity related insulin resistance and the obesity-associated increase in pro-inflammatory cytokine production (Pierce, Ballard-Barbash, et al. 2009). Non-diabetic women diagnosed with breast cancer who's insulin measurements were in the highest quartile, compared to those in the lowest quartile, had a 2-fold increased risk of recurrence and 3-fold increased risk of mortality (Goodwin et al. 2002). Insulin and inflammatory mediators have been associated with increased breast cancer cell growth and proliferation and increased adverse sex hormone levels (Rose, Komninou, and Stephenson 2004). Furthermore, obese individuals typically have a greater fat mass. Fat mass is a key site for aromatase enzyme activity which contributes to adverse/carcinogenic hormonal profiles through the conversion of androgens to oestrogens (Niraula et al. 2012). Excessive oestrogen production through this pathway is an established risk factor, and breast cancer mortality and morbidity are improved when this enzyme is inhibited.

Thus, the majority of evidence indicates that obesity and it's related metabolic and clinical implications is a significant risk factor for poorer prognosis in women who have been diagnosed with breast cancer. The next section of this chapter aims to discuss the implications of weight change after treatment, and how that may influence disease free and overall survival.

2.1.3 Change in body weight and BMI after a breast cancer diagnosis

Research from the last two decades has shown a consistent trend of body weight gain occurring in 50 to 100% of women who are completing or have completed adjuvant treatment for breast cancer; reviewed here (Rooney and Wald 2007, Harvie 2010). Earlier studies indicated gains of up to 11kg during and soon after chemotherapy (Camoriano et al. 1990). However, more recent studies indicate this figure has diminished, with 5kg gains still common during the same time frame (Irwin et al. 2005, Ingram and Brown 2004, Harvie et al. 2004, Demark-Wahnefried, Campbell, and Hayes 2012). This decrease in weight gain may be related to increased awareness of the importance of a healthy lifestyle post treatment amongst oncologists and patients alike (Demark-Wahnefried, Campbell, and Hayes 2012). It was also noted that the probability of re-attaining the pre-diagnosis weight was inversely associated with initial post-treatment weight gains (Nichols et al. 2009). This suggests that early strategies to prevent weight gain are critical to long term management of the issue.

Body weight change, recurrence and survival after breast cancer treatment

A number of studies in large populations (range of n=111 to12915) (Chen et al. 2010, Caan et al. 2012, Nichols et al. 2009, Kroenke et al. 2005, Zhang et al. 1995, Daling et al. 2001, Thivat et al. 2011) have shown inconsistent associations between body mass change and breast cancer disease free survival. However, large lifestyle intervention trials conducted over the last decade indicate that intentional weight loss through appropriate nutrition and exercise habits may elicit survival benefit for survivors of breast cancer (Ligibel and Goodwin 2012, Demark-Wahnefried, Campbell, and Hayes 2012). A large (n=693) 4-year long randomised controlled trial is currently underway in the USA to better understand this (the ENERGY trial: Exercise and Nutrition to Enhance Recovery and Good Health in You). This study is investigating the efficacy and feasibility of achieving sustained weight loss through group based cognitive-behavioural therapy in women who have been diagnosed with breast cancer in the last 5 years. Its aim is to examine the impact of weight loss on recurrence, disease free survival, quality of life and co-morbidities (Rock et al 2013).

Four large prospective cohort studies (Kroenke et al. 2005, Caan et al. 2006, Chen et al. 2010, Nichols et al. 2009) and a project combining four large data sets (Caan et al. 2012), have revealed an increasingly consistent U-shaped trend for body weight change and mortality in women after completion of treatment for breast cancer. Kroenke et al (2005) reported findings from the Nurses' Health Study data set (N=5204), which indicated that non-smoking women of normal weight at diagnosis (<25kg/m^2) who had an increase in BMI of more than 2kg/m^2 after diagnosis experienced increased risk of total mortality, breast cancer recurrence and mortality (Kroenke et al. 2005). In addition, for those who experienced a change in BMI of 0.5 to <2kg/m^2, a significant increase in total mortality and breast cancer recurrence was noted, while a positive trend was seen for breast cancer mortality (Kroenke et al. 2005). Relationships were not significant for women who smoked, and were weaker for postmenopausal as opposed to premenopausal women (Kroenke et al. 2005).

In contrast to these findings, Caan et al (2006) reported findings from the Life After Cancer Epidemiology (LACE)(Caan et al. 2008) data set (N=1692), which were previously combined with the control group of the Women's Healthy Eating and Lifestyle (WHEL) study (N=3215)(Caan et al. 2006). Compared to those who maintained weight, total mortality and recurrence for those who gained weight of any magnitude, or those who experienced moderate weight loss were not significantly different. Of note, there were no significant differences found between smokers and non-smokers. However, compared to women who maintained weight, those who lost more than 10% of their pre-diagnosis body weight experienced a significant increased risk of breast cancer

recurrence (HR = 1.7, 95% CI: 1.0 to 2.6) and death due to any cause (HR = 2.1, 95% CI: 1.3 to 3.4) (Caan et al. 2006).

Nichols et al (2009) (Nichols et al. 2009).(N=3993), Chen et al(Chen et al. 2010) (N=5042) and 'The After Breast Cancer Pooling Project' (N=12 915) (Caan et al. 2012), which combined four large prospective data sets (Chen et al. 2010, Kroenke et al. 2005, Caan et al. 2008, Pierce et al. 2002), have reported that the lowest mortality risk is found in those who maintained their weight within 2-10% of their body weight taken at diagnosis.

Nichols et al (Nichols et al. 2009) reported that compared to those women who maintained their weight (a change of $\pm$2kg), women who gained >10kg from the time of diagnosis experienced a 70% increased risk of mortality. In addition, an increased risk of mortality was also reported for those who lost 2.1 to 10kg (HR = 1.39, 95% CI: 1.04 to 1.86), and greater risk again for those who lost more than 10kg (HR = 2.66, 95% CI: 1.73 to 4.07)(Nichols et al. 2009). Similar results were found in a Chinese population, where a body weight loss of >1kg was seen to increase the risk of mortality (HR: 2.41; 95% CI: 1.62–3.58) (Chen et al. 2010).

A recent collation of four large prospective data sets including North American and Chinese studies indicated a non-significant increase in death following weight gain after treatment, yet confirmed a 40% increased risk of death for US populations who lost >10% of their pre-diagnosis weight in the 18 to 48 (median: 25) months following (Caan et al. 2012). Of note, in those who lost >10% of their weight, overall mortality risk for the Shanghai cohort was more than two-fold the risk seen in the US population (Shanghai: HR, 3.25; 95% CI, 2.24–4.73 versus US: HR: 1.41; 95%CI, 1.14–1.75). Importantly, non-breast cancer mortality was increased with those who lost weight (HR: 1.62; 95% CI, 1.21–2.19) in US populations only, however for Shanghai, 86% of the deaths were due to breast cancer related events, and HR for total and breast cancer mortality were of similar magnitude (HR, 3.60; 95% CI, 2.39–5.42).

Additionally, sequelae of body weight change after treatment extend past breast cancer related events. Increasing gains and losses in body weight have been associated with increased risk of CVD (Nichols et al. 2009), and arthralgias secondary to aromatase inhibitors (Demark-Wahnefried, Campbell, and Hayes 2012). Weight gain alone has been associated with increased surgical complications, lymphoedema risk, fatigue & menopausal symptoms, while weight loss alone has been associated with poorer bone health and fracture risk (Demark-Wahnefried, Campbell, and Hayes 2012).

Vast differences in timing of recruitment, assessment of weight (self-report and clinician measured) and definition of breast cancer recurrence may account for the differences in results between these large studies.

Summary of body weight and breast cancer survival

The weight of evidence currently indicates that maintenance of body weight following treatment is associated with the lowest risk of mortality, recurrence and co-morbidities. Significant loss of body weight is strongly associated with poorer mortality outcomes in prospective trials, while inconsistencies have been noted for body weight gain. A limitation of these studies is that none have accounted for the influence of body fat% or LBM change. Individual and combined changes in these different body compartments are likely to have a large influence on metabolic health, and therefore may be one reason as to why inconsistencies have been noted in cohorts to date.

2.1.4 Fat mass and LBM change and outcomes after breast cancer treatment

LBM growth typically accounts for 20-40% of total weight gains in disease free populations (Forbes et al. 1996). Simultaneous fat mass gain and loss of LBM is common after treatment for breast cancer (Rooney and Wald 2007). Studies of breast cancer survivors have shown that after chemotherapy, total fat mass gains of 2.4kg (Demark-Wahnefried et al. 2001) to 6.7kg (Harvie et al. 2004) were accompanied by LBM losses of -0.4kg to -1.7kg, respectively. Women who seemingly maintain their weight in the years after treatment still undergo these adverse changes, such that the loss LBM matches the increase in adipose tissue (Kutynec et al. 1999). Factors that are linked with greater increases in fat and LBM loss include premenopausal as opposed to postmenopausal status at diagnosis (Demark-Wahnefried et al. 2001), experiencing treatment related menopause (Goodwin et al. 1999), receiving chemotherapy compared to no chemotherapy (Harvie 2010), a lower BMI at diagnosis, and those who are least physically active after treatment (Irwin et al. 2003). Finally, the loss of LBM is still prevalent, albeit of smaller magnitude in postmenopausal compared to premenopausal breast cancer populations (Aslani et al. 1999, Harvie et al. 2004).

The most notable changes in body composition are seen during adjuvant chemotherapy and in the 6 to 12 months following this (Harvie et al. 2004, Demark-Wahnefried et al. 2001, Aslani et al. 1999, Battaglini et al. 2007), while increases in total weight still occur after this point (Nichols et al. 2009). It has been suggested that longer regimes of chemotherapy have resulted in greater weight increases. However, there are no studies that have reported weight change comparing the same agent at different durations (Gadéa et al 2011). Furthermore, smaller studies have indicated an increase in weight after longer (~6 month duration) cyclophosphamide, methotrexate, and 5-

flurouracil (CMF) regimens, but no weight change after shorter (~3 month duration) Adriamycin and cyclophosphamide (AC) (Gadéa et al 2011) regimens. On the other hand, larger trials have indicated significant weight gain irrespective of chemotherapy agent (Goodwin et al 1999; Saquib et al 2007; Makari-Judson et al 2007).

Loss of LBM with concurrent fat and total weight gains are associated with metabolic dysfunction including impaired glucose metabolism (Healy et al. 2010), high triglyceride levels (Hamilton, Hamilton, and Zderic 2007), and chronic inflammation in breast cancer populations (Mourtzakis and Bedbrook 2009). Cheney et al (Cheney, Mahloch, and Freeny 1994) noted that of those women who gained fat mass after chemotherapy, all experienced an increase in visceral adipose tissue measured by CT scan. Visceral adipose tissue is known to increase risk of metabolic syndrome related diseases though an increase in chronic inflammation (Lee et al. 2009). While it has been established that fat tissue is a very active endocrine and immune regulating tissue, skeletal muscle tissue may also have independent and/or complimentary influence on these regulatory pathways (Mourtzakis and Bedbrook 2009, Flynn, McFarlin, and Markofski 2007, Pedersen and Febbraio 2012). Thus a decrease in LBM could be an important marker of, and/or exacerbate the metabolic dysfunction associated with visceral adipose tissue deposition.

Additional considerations for measurement of body composition in breast cancer populations

Fluid accumulation as a result of lymphedema is an important consideration for measuring body composition in breast cancer survivors. Up to 33% of women who have completed treatment for breast cancer have an increased risk of developing treatment related lymphedema in the arm (Hayes et al 2008). Compared to women who are treated surgically with a lumpectomy, those who have undergone axillary lymph node dissection experienced significantly higher rates of lymphoedema (DiSipio et al 2013). In addition, greater risk is also associated with an increasing extent of axillary node dissection, and treatment with radiotherapy.

A recent review discusses strengths and weaknesses of common body composition measurement devices, and their suitability in a cancer survivor setting (Di Sebastiano and Mourtzakis 2012). The measures discussed included bioelectrical impedance analysis (BIA), skinfolds, dual energy X-ray absorptiometry, magnetic resonance imaging (MRI), computed-tomography scans (CT scans), under-water-weighing (UWW) and air displacement plethysmography (ADP).

BIA is strongly influenced by fluid changes in the body, thus its measure of LBM and fat mass may be confounded by lymphedema status (Di Sebastiano and Mourtzakis 2012). Similarly, skinfold assessment may be significantly affected by upper limb swelling, particularly the triceps and biceps

measures. Accumulation of fluid may acutely alter the thickness of a skinfold, which would significantly reduce the reliability of the measure. DEXA, magnetic resonance imaging (MRI) and computed-tomography scans (CT scans) allow segregation of body compartments, including those with increased swelling. However, unlike DEXA which allows specific assessment of limbs and trunk, MRI and CT scans normally measure body composition from a single picture, or 'slice', typically at the level of the lumbar spine, and not allow interpretation of body composition for the upper limb (Di Sebastiano and Mourtzakis 2012). Measurement via UWW and ADP, which utilise the principles densitometry, may be altered as arm volume increases as a result of fluid retention. ADP & UWW calculates body composition by using established densities of LBM and fat mass, thus a change in density due to water retention may make the measure less reliable. For measurement using ADP, a previous study has shown that consumption of over 1000ml of water has the effect of increasing the value for fat mass without altering the LBM result (Vukovich and Peters 2003). However, the same study indicated that fluid retention as a result of creatine loading was reflected by an increase in LBM. A later study indicated that consumption of a small amount of food or water (cereal and milk or 350ml of Gatorade, respectively) resulted in no significant change to body composition when measured by BIA, DEXA or ADP (Heiss et al 2008). Therefore, a change in arm volume of 1000ml as a result of swelling may influence body composition analysis, and it may be less likely to affect LBM results. Alternatively, the gradual accumulation of fluid in the interstitial space may be interpreted like creatine-induced water retention. The effect that lymphoedema-related swelling has on body composition measurement is currently not known and should be accounted for in studies using densitometry.

2.1.5 Mechanisms of body composition changes after treatment for breast cancer

Even with detailed assessments of energy balance (physical activity, total energy intake, resting energy expenditure), the body weight increases seen in breast cancer survivors are above those predicted in the energy balance equations. Two studies have noted that weight gain has been of significantly greater magnitude compared to predicted weight changes (Demark-Wahnefried et al. 1997, Harvie et al. 2004). In fact, one study reported body weight gain when the population reported lifestyle parameters predictive of a negative energy balance (Demark-Wahnefried et al. 1997). Physical activity levels after treatment have been better associated with body composition changes after treatment. Breast cancer survivors have repeatedly reported stable (Harvie et al. 2004, Kutynec et al. 1999, Irwin et al. 2003) or lower levels of physical activity during and after treatment, and these reductions have been correlated to increases in weight (Irwin et al. 2005, Demark-Wahnefried et al. 2001, Goodwin et al. 1999, Kumar et al. 2004). However, limited data is available to explain the loss of LBM (Demark-Wahnefried et al. 1997).

Drawing from other populations, chemotherapy induced myotoxicity (Fanin et al. 2000) and increased systemic inflammation (Mourtzakis and Bedbrook 2009) after treatment may be two factors that explain LBM loss in breast cancer populations. Both of these factors require further investigation in future studies. Currently, it is thought that loss of LBM is greater in those who have completed chemotherapy (Sheean, Hoskins, and Stolley 2012), while gains in LBM have been noted in post-menopausal women prescribed aromatase inhibitors (AIs), due to a greater androgenic hormonal profile (van Londen et al. 2011).

2.1.6 Summary of body composition and breast cancer

Collectively, the current literature indicates that greater waist girth or WHR is associated with premenopausal breast cancer risk, while postmenopausal risk is associated with a higher BMI. Regardless of menopausal state, greater BMI or waist girth at diagnosis are equally associated with increased risk of breast cancer related morbidity and mortality. For both lines of investigation, no definitive studies have indicated a role for LBM in breast cancer risk or prognosis.

After diagnosis, a large literature base indicates that weight stability and/or moderate weight loss (<3kg) is associated with the lowest risk of morbidity and mortality from any cause, while both body weight gain and significant body weight loss are associated with poorer outcomes.

More detailed analyses of change in LBM and fat mass following treatment for breast cancer may provide a better understanding of how body weight change relates to mortality and morbidity outcomes. Gain in general and abdominal fat mass is associated with greater inflammation (Mourtzakis and Bedbrook 2009, Dee 2010), greater risk of cardiovascular disease (Pierce, Ballard-Barbash, et al. 2009), and risk of metabolic syndrome (Healy et al. 2010). There is a dearth of long term evidence in regards to modifiable factors that predict LBM after treatment, however a significant number of shorter exercise and nutrition interventions (see next section) indicate longer term data is warranted.

2.2 Exercise and dietary interventions and their effect on body composition in breast cancer survivors

2.2.1 Controlled trials evaluating the effect of exercise alone

Exercise training and body composition has been extensively researched in breast cancer populations. An outcome of body composition change has been reported in 31 controlled exercise

trials. Of these, five (n=612; range: 20-242) have been conducted during adjuvant chemotherapy (Battaglini et al. 2007, Winningham et al. 1989, Segal et al. 2001, Courneya et al. 2007, Mutrie et al. 2007), two (n=130; range: 30-100) have spanned both adjuvant chemotherapy and after completion of treatment (DeNysschen et al. 2011, Nikander et al. 2007), and 24 (n=2389; range 16-573) have been conducted after completion of adjuvant therapies excluding endocrine therapy (Ligibel et al. 2008, Daley et al. 2007, Mustian, Katula, and Zhao 2006, Schmitz et al. 2009, Irwin, Alvarez-Reeves, et al. 2009, Matthews et al. 2007, Schmitz, Ahmed, et al. 2005, Herrero et al. 2006, Burnham and Wilcox 2002, Pinto et al. 2005, Rogers et al. 2009, Courneya, Mackey, et al. 2003, Courneya, Friedenreich, et al. 2003, Vallance et al. 2007, Basen-Engquist et al. 2006, Saarto et al. 2011 , Twiss et al. 2009, Winters-Stone et al. 2011, Fernández-Lao et al. 2013, Mulero Portela et al. 2008, Nicole Culos-Reed et al. 2006, Guinan et al. 2013, Pinto et al. 2003, Nuri et al. 2012). Change in total body weight and/or BMI was reported in 29 studies, a measure of body fat (percentage or fat mass) was reported in 18 studies, two studies reported change in sum of skinfolds without conversion to body fat%, while LBM was reported in 14 studies. DEXA was used in 8 studies, BIA was used in five studies, sum of three skinfolds was reported in five studies, and sum of five skinfolds was reported in 3 studies. Studies reporting a measure of LBM are summarized in Table 2.1

Body composition changes in controlled exercise interventions

Description of studies

Of the 29 studies that reported total weight changes as a result of an exercise intervention, 27 found no change in total body weight. In contrast to the minimal changes seen in overall body weight, nine (Courneya, Friedenreich, et al. 2003, Herrero et al. 2006, Burnham and Wilcox 2002, Schmitz, Ahmed, et al. 2005, Irwin, Alvarez-Reeves, et al. 2009, Fernández-Lao et al. 2013, Battaglini et al. 2007, Winningham et al. 1989, Courneya et al. 2007) of 17 indicated a reduction in body fat%. In addition, two (Guinan et al. 2013, Fernández-Lao et al. 2013) of four of studies reported a reduction in waist girth when compared to control. Seven of the 13 studies that reported body composition as a primary measure reported a significant improvement in body fat% favouring the exercise groups (Herrero et al. 2006, Burnham and Wilcox 2002, Irwin, Alvarez-Reeves, et al. 2009, Battaglini et al. 2007, Schmitz, Holtzman, et al. 2005, Winningham et al. 1989, Fernández-Lao et al. 2013), with one showing a trend for benefit (Courneya et al. 2007) the five remaining studies reported no differences in body fat% change between groups (Matthews et al. 2007, Nuri et al. 2012, Guinan et al. 2013, DeNysschen et al. 2011, Winters-Stone et al. 2011)

In regards to studies that reported change in LBM, six of 10 (N=798, range: 16-242) studies reported a significant improvement in LBM for exercise groups compared to control (Herrero et al. 2006, Battaglini et al. 2007, Schmitz, Ahmed, et al. 2005, Courneya et al. 2007, Irwin, Alvarez-

Reeves, et al. 2009, Fernández-Lao et al. 2013). Of the four studies that have reported LBM change using DEXA, ADP and UWW three studies reported greater gains in LBM for the exercise group (Courneya et al. 2007, Schmitz, Ahmed, et al. 2005, Irwin, Alvarez-Reeves, et al. 2009), while one study indicated no effect for exercise (Matthews et al. 2007). A more detailed explanation of why these differences occurred can be found in the following sections.

Timing of exercise, i.e. during/after treatment, and type of exercise training aerobic or resistance exercise and supervised or home based, are important mediators in body composition changes and therefore require a more in-depth investigation, discussed below. Studies reporting LBM as a primary outcome and/or used a high quality measure for body composition will be the focus of this review. See Table 2.1 for a summary of these trials reporting high quality body composition data.

Effects of exercise on body composition during chemotherapy

The majority of studies evaluating the effects of exercise on body composition during chemotherapy have shown a benefit for both LBM and BF%. One trial has indicated no change (DeNysschen et al. 2011) compared to controls. However, the majority of studies investigating the effects of exercise training during chemotherapy have reported increases in LBM(Courneya et al. 2007, Winningham et al. 1989, Battaglini et al. 2007) and attenuated gains in body fat% (Courneya et al. 2007, Winningham et al. 1989, Battaglini et al. 2007). While current literature in healthy populations would indicate better LBM maintenance to be associated with resistance training, both aerobic and resistance training interventions have had mixed results during chemotherapy.

Aerobic exercise alone

Aerobic exercise prescription during chemotherapy has had mixed effects on LBM and other markers of body composition. Denysschen et al (DeNysschen et al. 2011) found no effect for aerobic exercise on body composition during chemotherapy. However, the study was confounded by their control group, who performed the same amount of exercise as both intervention groups. Similarly, a high quality study by Courneya et al (Courneya et al. 2007) using DEXA noted aerobic exercise training alone tended to decrease body fat% gains, while having no significant effect on LBM. However, further analyses in the study by Courneya et al (2007) revealed that compared to control, significant decreases in body fat% and increases in LBM were noted for exercisers with later stage disease (IIB/IIIA), while no effect was found for earlier stage disease (I/IIA). The reason for differing results in those with later stage disease is currently unknown, however a potential explanation may be related to treatment associated inflammation (Mills et al. 2008). Those with later stage disease undergo more intensive treatments, which in turn are related to higher levels of inflammation and myotoxicity (Mills et al. 2008). Higher levels of inflammation are known to

induce proteolysis through up regulation of the ATP-ubiquitin-proteasome pathway (Debigare, Cote, and Maltais 2001).

Since loss of LBM may be higher in those with later stage disease, an intervention that reverses LBM loss may create a greater 'control versus intervention' difference than if the effect of intervention is compared to those with a smaller loss of LBM. For example, those diagnosed with earlier stage disease experience less intensive treatment and less inflammation (Mourtzakis et al 2009), therefore they may experience a smaller LBM loss, thus creating a smaller disparity between control and intervention groups. In addition, baseline levels of fat and total body mass may influence the effect of the intervention, i.e those with a higher body fat may experience a greater loss of body fat than leaner individuals. Unfortunately, raw data on absolute LBM and fat mass values were not given (Irwin et al 2009) so it is not able to be determined if the changes reported were due to differences at baseline.

TABLE 2.1 CONTROLLED EXERCISE TRIALS EVALUATING LBM CHANGES IN BREAST CANCER SURVIVORS

Author, Yr, Design, Country Rank, Quality, Primary outcome	**Population**	**Intervention**	**Body composition and adherence outcomes**	**Comments**
Exercise interventions during chemotherapy				
Winningham, 1989 RCT – 2 arm USA NHMRC: II Quality: + Body composition	N=34 initial; N=24 follow up; Post Sx; Age: 45.6yrs (32-66yrs); Menopausal status: 42% postmenopausal No significant differences between groups	10-week intervention during CTx INT: 3 sessions/wk, aerobic exercise: 20-30min/session, 60-85% of highest HR. Measures: skinfolds (3 sites): Supra-iliac, anterior thigh, triceps. Weight, Height. Max VO2, Obesity defined: >30% fat as per fold measures.	No significant changes in body weight (kg) INT: +0.88kg, CON: +1.99kg (F=1.86, p=1.888) Significant ↓ in body fat% INT: -0.51%, CON +2.19%; F=5.26, p=0.033 Trend for ↑ in LBM (kg) INT: +2.04kg, CON: -1.26kg, P=0.066 INT: Stabilised body fat%; CON: ↑body fat%. Obese individuals increased body fat% 2-fold compared to non-obese	Initial fat loss occurred in upper body. Limitations: Small sample size, prednisone use, no dietary control. Obese individuals in CON gained more weight Limited demographical information
Battaglini, 2007 RCT, 2-arm USA, Brazil NHMRC II Quality: +/O Body composition	N=20; Age: 57yrs; Wt: 82.2kg (SD 25kg); Fat mass: 30.1% (SD 4.2) No other demographical results reported Sig diff between groups not reported	Duration: 15 weeks; INT: 6 wks post-Sx. INT: 2/wk for 60mins. 48-84hrs apart. 40-60% of both AET and RET. 6-12min cardio, 15-30min RET (6-12 reps) CON: instructed not to engage in exercise Measures: skinfolds (3 sites)**:** triceps, supra iliac, abdomen. Fat and lean mass VO_2, Muscle strength (est. 1-RM) through a series of machine tests	Body weight not reported Significant ↓ in body fat% between groups INT: -3.1%, CON: +1.1%, P=0.004 Significant ↑ in %LBM between groups INT: +3.1%, CON: -0.2%, P=0.004 between groups (NS within groups for either LBM% or Body fat %) Adherence: 100%	As treatment progressed, group differences increased. Results made significant by the deterioration in control group and improvements in INT group. Minimal demographical information
Courneya 2007 RCT, 3-arm Canada NHMRC: II Quality: + Body composition	N=242, Age: 49yrs; Stage I-IIIA, 100% during chemotherapy; Menopausal status: 33% postmenopausal; Taxane CTx: 34.1% No sig diff between groups across any parameters	Mean duration: 17 weeks (duration of CTx) AET: 3 sessions/wk on cycle, treadmill or elliptical aerobic exercise @60% to 80% of VO_{2mac}. 15 increased to 45 minutes by end of INT. RET: 3 sessions/wk, 2 sets of 8-12 reps @ 60-70% of est. 1-RM. 9 exercises CON: Asked not to initiate an exercise program for the duration Measures: Wt, Ht, DEXA, Arm water displacement, QOL, VO_{2max} (treadmill) Strength: 8-RM bench and leg extension	No significant change in body weight AET: +1.0, RET: +1.6, CON: +1.2, P=0.698 Trend for ↓ body fat% for AET vs. CON* AET: +0.2, RET: +0.3, CON: +1.0, P=0.076* Significant ↑ in LBM (kg) for RET vs. CON AET: +0.5, RET: +1.0, CON: +0.2kg, P=0.015 (b/w RET & UC) Body comp when assessing only Stage IIB/IIIA. Significant ↑ in LBM (kg) for RET & AET AET: +1.3, RET: +2.6, CON -0.3, RET>AET>CON (all significant P<0.007); Significant ↓ in body fat% for AET & RET AET: -1.0, RET: -1.4, CON: +1 RET vs. CON, P=0.019. AET vs. CON, P=0.034 No significant changes for Stage I/IIA for LBM or body fat%.	RET more effective for LBM increases in all populations. AET more effective overall for body fat% loss. Any exercise is beneficial for body fat% in later stage disease. RET is more effective for LBM in those with later stage disease AET improves VO_{2max} compared to RET & UC RET improves strength compared to AET & UC

Exercise interventions during and after treatment				
Denysschen, 2011 RCT 3-arm USA NHMRC: II Quality: O Body composition	N=100 (BrCa, Female 100%); Age: mean for groups 48.7yrs to 51.6yrs; Stage I-III; BMI: 25.2 (5.5); Menopausal status: 34 to 44% premenopausal; During CTx: 100% Differences at baseline: LBM higher in non-exercising control group; More smokers in control group	Duration: During treatment (During): 4-6 months; Post-treatment (Post): 4-6 months; Home based with phone calls. Ppt choice of aerobic exercise; 3-5 sessions/wk. 20-30 min @ >12-14 RPE. Goal: 1200 to 1500kcal/wk expenditure Intervention groups: EE: Exercise during and after treatment CE: Exercise after treatment only CC: No exercise (control) Measures: DEXA, Wt, Ht Max VO_2 – treadmill, Karnofsky, Nutrition symptoms checklist	No significant change in body weight (all values are net change from baseline value) During: EE: -1.1kg, CE: +2kg, CC: -2.1kg Post: EE: -0.6kg, CE: +2kg, CC: +2.7kg No significant change in LBM (CC had higher LBM at baseline than EE) During: EE: +1.8kg, CE: +1.3kg, CC: -0.8kg Post: EE: +0.3kg, CE: +0.6kg, CC: -0.6kg No significant changes in body fat% During: EE: +0.5%, CE: +1.2%, CC: +1.5% Post: EE: +1%, CE: +1.5%, CC: +2.8% Adherence: EE group: 74% @ end of CTx, 78% @ end of Study; CE group: 86% at end of study.	Trend for more fat gains in CC group. Did not measure control group activity and exercise prior to study was measured by self-report. CC group was shown to exert the same MET-hrs each week as CE and EE. Dietary intake not measured. Significant differences in baseline LBM (EE vs. CC)
Aerobic Exercise interventions after surgery, radiation therapy and chemotherapy				
Irwin 2009 RCT 2-arm USA NHMRC II Quality: + Primary outcome (body composition)	N=75, Age: 56.5yrs; Time since Dx: 3.3yrs; Stage 0-IIIA. Menopausal: 100% postmenopausal. Sedentary Excl: Smokers, T2DM and previous cancer No sig differences in baseline parameters	Duration: 6 month (subset 12 months, n=48) INT: 150min/wk aerobic exercise, 5 x 30min sessions/week. 3 supervised, 2 unsupervised. 60-80% of HRmax. Any aerobic apparatus. Any resistance training/yoga did not count toward PA goals. CON: Allowed to exercise but received no support until after trial. Measures: DEXA, Ht, Wt, Waist, Hip 7-Day Physical Activity Log, FFQ for dietary intake	No significant changes in body 6 months: INT: -0.55kg, UC: +0.1kg, P=0.39. 12 mth: INT: -0.2 vs. UC: +0.6, P=0.61 Significant ↓ in body fat% @ 6 & 12mth 6 mths: INT: -0.79%, CON: +0.42%, P=0.0022 12mths: -1.19%. -0.03%, P=0.043 Significant ↑ in LBM @ 6 but not 12 mth 6 mth: INT: +0.34kg, CON: -0.35kg, P=0.047 12mths: INT: +1.7, CON: +0.2, P=0.25 No change in waist (cm) INT: -1.5, CON: -0.8, p=0.57 No change in hip girth (cm) INT: -0.8, CON: +0.6, p=0.21 Significant LBM changes for those <56yrs. Significant body fat% ↓ for: >56yrs, Later stage, BMI <30. Increased exercise related to increased change.	Supervised exercise may increase LBM and ↓ body fat%, with larger effects in certain demographics. Greater adherence to exercise is correlated to increased body fat% changes.
Matthews 2007 RCT, 2-arm, Multicentre USA NHMRC: II Quality: + Body composition	N=34; Age: 51.3 to 56yrs (means of groups); Stage I-III; Time since Dx: 0.7yrs; Menopausal status: 100% postmenopausal, BMI >25, No sig diff between groups @ baseline	Duration: 12 week INT: Home based, ↑ walking from 3 x 20-30min session, to 5 x 30-40min sessions. RPE 11-13. Pedometers given out. Contact: 1x 30min home face-to-face. 5 follow up phone calls. CON: Continue normal activities Measures: DEXA, BIA, Wt, Ht; Physical Activity: CHAMPS questionnaire + Accelerometers Dietary intake: DHQ and NCI F&V	No significant change in body weight (kg) INT: 0.04 vs. 0.01, P=1.00 Trend for ↓ in body fat % INT: -0.2; CON: +0.4, P=0.15 No significant change in LBM (kg): INT: +0.21, CON: -0.3, P=0.27 Volume of exercise and fruit and vegetable consumption had no bearing on change Walking increased: 4.9 to 16.8MET-hrs/wk, compared to 5 to 6.6 in control group. Adherence: 97% at 6 weeks; 71% at 12 weeks	Physical activity was increased. Trends for body composition changes were present. Longer duration study may show results RPE used to determine aerobic work.

Burnham, 2002, 3-arm RCT NHMRC IIQuality + Primary (body composition)	N=18 (BrCa n=15) All groups contained BrCa (n=5) and Colon Ca (n=1) Age: means 50 to 56yrs; Time post Rx: 9 to 10.3mths No sig differences for bline variables	10-week intervention 3 sessions/wk, mixed aerobic modalities; Duration: 14min week 1 to 32min at wk 10. ModINT: Initial 40-50% of HRR, final 60% of HRR LowINT: Initial 25-35% of HRR, final 40% of HRR. Measures: body fat% from skinfolds (3 sites), Wt, VO2 Peak (treadmill); QOL; Flexibility	Exercise groups combined for final analysis No significant change for body weight INT: -0.2%, CON: -0.6%, P>0.05 Significant ↓ in body fat % INT: -2.4%, CON: 0.1%, P<0.05 Exercisers had significant increase in absolute and relative VO2 peak compared to controls, P<0.05 Attendance for exercise: 95%	Both low and moderate intensity exercise offers benefit in a number of physiological and psychological areas. Limited by small numbers, and short duration and somewhat heterogeneous sample.
Guinan, 2013 2-arm RCT NHMRC II Quality: 0 Primary anthropometrics and blood biomarkers for metabolic syndrome	N=26, Age: 48.12yrs, 30.8% postmenopausal, 96.2% Caucasian, Time since CTx: 3.74 months, Stage I/II/III: 26.9/50/23.1%; Tamoxifen: 57.6%, AI: 19.2%. No diff between groups	8-week intervention, 2 supervised sessions/wk, building from 1 to 5 home sessions/wk. AET: 35-65% HRmax (lower range for those with lower initial fitness). HR monitor for adherence in gym and at home. 5% increase in aerobic intensity each 2 weeks. CON: Education session on exercise Measures: 0, 8 & 12 weeks Body composition: BIA & waist PA: GT3X accelerometer Bloods: CRP, TChol, HDL, LDL, TG, BGLs, Insulin, HOMA-IR, HbA1c	ITT analysis: No change in weight, BF%, LBM or Waist at 8 or 12 weeks Confidence intervals for waist indicated change, but were not significant between groups. Per protocol analysis (>90% of exercise class attended) No reported change in weight, BF% or LBM Significant reduction in Waist girth at 3 months INT: -4.63 95% CI: -5.7 to -3.6; CON: -0.3 (-2.3 to 1.7), p=0.05. CRP: Within group reductions for INT, but no differences between groups at 8 weeks (p=0.07) and 12 weeks (p=0.69) Accelerometer did not show change in activity for per protocol or ITT. Godin activity questionnaire indicated an increase in 'total weekly exercise' for adherers (p=0.005)	Differences only found in those who adhered to the protocol >90%.
Resistance exercise interventions after treatment				
Schmitz, 2009, RCT, 2 arm Multicentre Quality: + NHMRC: II Secondary outcome (body composition)	N=141 @ baseline, N=130 final Past or current diagnosis of stable lymphoedema. Age: 56yrs, Time since Dx: 6.5yrs (1 to 15 yrs); Stage: I/II/III: 46%, 1%, and 31%. (n/a: 21%). Menopausal status: not reported. Sedentary. Baseline differences in tamoxifen (p=0.008). No other differences between groups	During: 12 months. INT: Progressive resistance training – 2 sessions/wk (90min per session) – 9-10 resistance exercises. Building 1 to 3 sets per exercise for 10 repetitions. 13 weeks supervised. 39 weeks unsupervised. No limit to weight increase. Lymphoedema flare up resulted in return to lowest available weight for upper body resistance. CON: Continue normal activity Measures: DEXA – LBM and Body fat % Wt, Ht, Arm volume. PA (MET min/wk); Diet	No significant changes in body weight INT: -1%; CON: -0.4%, P=0.47. No significant changes in body fat% INT: -0.3%; CON: -0.1%, P=0.19. No significant changes in LBM INT -1%; CON: -1.1%, P=0.67. No sig differences in lymphoedema status 1-RM Strength increased for INT group Bench press: INT: +29.4%, CON: +4.1%, P<0.001; Leg press: INT: +32.5%, +7.6%, P<0.001 Attendance at 3 monthly intervals: 96%, 88%, 81% 75%.	Weight lifting is safe in breast cancer populations: INT had ↓ severity of arm and hand symptoms, ↓ no. of exacerbations; ↑ muscular strength. Lymphoedema may have reduced expected strength gains.

Schmitz, 2005 + O'Hira, 2006 RCT, 2 arm Wait list control USA Quality: + NHMRC: II Safety of RET & Body composition	N=85, Age: 53.3yrs, Time since Dx: 1.7 to 2.01yrs, Time since last Rx: 1.1 to 1.2yrs, BMI <40kg/m²; Stage 0-IIIA; Menopausal status: 85% post. Sedentary No significant differences between groups	Duration: 6 months INT: 13 weeks; 2/wk resistance training. Supervised, then 13 weeks not supervised. 8-10 reps, 2-3 sets per exercise, 9 exercises for upper and lower combined No changes to aerobic CON: Wait list control, performed INT from 6-12 mths Measures: DEXA, Wt, Ht, Waist Bloods: IGF-axis, Insulin, HOMA DHQ, PAR-Q, 1-RM, QOL: CARES-SF Injury, Depression: CES-D	No significant change in body weight (kg) INT: +0.3, CON: +0.2, P=0.84 Significant decrease in body fat% b/w groups INT: -1.14, CON: +0.25, P=0.03 Significant increase in LBM (kg) b/w groups INT: +0.88, CON +0.02, P=0.008 No significant change in waist girth INT: -1.1%, CON: +0.22, p=0.08 No sig differences in lymphoedema status 1-RM Strength increased for INT group Bench press: INT: +63%, CON: +12%, P<0.001; Leg press: INT: +38%, +9%, P<0.001 Biochemical: No change for: Glucose, Insulin, HOMA, IGF-I, IGFBP-1, 2; Adherence: 0-6 mths: Mean 92%; Median: 96%; 6-12mths: Mean: 66%; Median: 77%	Resistance training may decrease body fat% and increase LBM. Strength gains much higher than tissue growth. Correlations for LBM and QOL: r=0.26, P<0.05; and Bench press and QOL: r=0.53, P<0.01.
Winters-Stone, 2011, RCT, 2-arm, USA Quality: + NHMRC: II Primary outcome: LBM, Fat mass, Bone mass, BMD	INT, CON N=52, 54; Postmenopausal: 100%; Age: 62.3, 62.2yrs (1.7); BMI: 29.5 (5.8); Time since Dx: 4.75yrs, 5.25yrs; Stage: O/I/II/IIIa: 7.7%/ 38.5%/48.1%/1.9%; 3.7%/40.7%/35.2%/9.3% Recevied CTx: 61.5%, 59.3%; RTx: 92.3%, 83.3%; AI: 42.3%, 40.7%; No diff b/w groups	INT: Supervised 2/wk Home based 1/wk for 1 year. Supervised: Free/machine/body weights 1-3 sets, 8-12 reps 3-4 upper body & 3-4 lower body exercises + 1-2 sets of 10 reps: 2 footed jumps 30cm in height. Home: Same as above with therabands CON: Whole body stretching – seated or lying **Measures:** 0, 6, 12 months DEXA: LBM, Fat mass, BMD Bloods/Urine: Osteocalcin, & urinary deoxypyrodinoline cross-links. Physical Activity: CHAMPS PA quesionairre Dietary intake: Energy + Calcium - Block FFQ	All b/w group differences No significant % change in body weight (kg) INT: +1.19%, CON: +0.27%, P=0.55 No significant % change in bone free LBM (kg) ITT INT: +1.38%, CON: +1.12%, p=0.91 INT group taking AIs had a greater LBM increase than those in INT group not taking AI. No effect for AI vs. Non-AI in CON group. No significant % change in body fat% INT: 0.00%, CON: -0.52%, P=0.50 No change in energy intake Exercise adherence: Total, Supervised & Home INT: 57%, 76%, 23%; CON: 62%, 72%, 44%	Greater increases in LBM for AI takers than non-takers in POWIR group. Small decrease in non-AI users in FLEX, vs. no change in AI takers. Resistance and jumping maintained spine BMD. Whereas CON group experienced decreased BMD.
Combined aerobic and resistance training after treatment				
Herrero 2006, RCT 2 arm Spain NHMRC: II Quality: + Body composition	N=16; Age: 50.5yrs; Time since Dx: 24-58 mths (range); Menopausal status: 100%; Stage I-II No differences across groups for any variables	Duration: 8 weeks; INT: 3/wk x 90min: 11 resistance exercises 3 sets for large and 2 for small groups. Progressive o/load; Aerobic: 20min 70%HRR, to 30min 80% HRR (or split: 2x10min). CON: Asked to not exercise. Measures: skinfolds (3 sites), Wt, Ht. Max VO_2, 100-110% BM lift; Endurance, EORTC, Sit-stand	No significant change in body weight (kg) INT: -1.1kg, CON: -0.4, 95%CI: -2.93 to 1.38 Significant ↓ in body fat % INT: -2, CON: 0; 95%CI: -3 to -3.8, p<0.05 Significant increase in LBM (kg) INT: +0.7, CON: -0.3, 95%CI: 0.25 to 1.86 Significant ↑ in sit-to-stand, leg press and VO_{2max} for INT, P<0.05; Trend for bench press: P=0.08 Adherence to training: 91%	Relative ↑ in LBM and ↓ body fat% in a short time period. INT was 270 minutes/wk over 3 sessions. Increased volume may explain changes. Significant improvement in VO_{2mac}, lower body function and strength. Small sample size, skinfolds and limited demographical information given

Fernandez-Lao, 2012 3 arm –wait list control Spain Quality: O NHMRC: III-1 Pseudo-randomised	N=96, Age: 48, Time post Rx: 75% <12 mths since; Stage: I-IIIA: Tamoxifen: 39, AI: 39, None: 8	Land-based intervention (Land) 60min, 3/wk 8 weeks AET: 40-50% of session <60% HRmax RET: 2-3 sets, 8-12 repetitions DVD of exercises provided after the program Water based intervention (Water) Same as above with water related restrictions on exercise CON – given reading material for breast cancer related exercise and nutrition. No caloric restriction Measures 8-point BIA, Wt, perometry of arm, waist, QOL (EORTC-QLQ-BR23)	Group x time interaction for LBM: F=3.566; p=0.008 Land had greater increases than Water (p<0.001) & CON (p=0.009) Group x time interaction for body fat%: F=3.376; p=0.011. Land had greater decreases than Water (p<0.001) CON (0.002) Group x time interaction for waist girth: F=4.553, p=0.002. CON had greater increases than Land (p<0.001) or Water (p-0.003) Group x time interaction for breast symptoms: F=9.048; p<0.001. Water greater increases than Land (p<0.01) or CON (p<0.05). Adherence: Land: 84.8% vs 91.9% water	Those who exercised maintained body weight, while those who did not exercise gained weight. Difficult to control resistance and consistency in the water.
Saarto 2011 2-arm RCT Finland NHMRC: II Quality: + Bone loss	N=573; Age: 35 to 68. Invasive breast cancer; Time since end of adj. RTx or CTx or started endocrine Rx <4mths earlier.	12 months, 1 supervised + 2-3 home sessions Aerobic step class: 150 to 180 jumps (10cm to 15 then 20cm benches after 8 mths) Circuit training: Aerobics – Circuit class: 150 to 180, with only 100 more demanding jumps in later stages Classes aimed at 14-16 RPE Home sessions: 100 leaps + Nordic walking/walking CON: Normal activity with no formalised supervised or home sessions Measures: Body comp: DEXA, Wt, Ht CV fitness: 2km walk test; Dynamic strength: figure 8 test Leisure time PA – 2-wk prospective exercise diary	No significant difference in weight change. Premenopausal: Body weight increase 1.4 to 1.9% with no training effect Postmen: No change in weight No significant effect for LBM Premenopausal INT: 43.7 → +0.3kg (0.077 to 0.617) NS CON: 44 → +0.268 (-0.041 to 0.0577) Post Menopausal INT: 44.6 → +0.34kg (-0.005 to 0.694) p=0.13 CON: 43.2 → -0.011 (-0.288 to 0.266) No significant change in fat mass between groups Premenopausal 3.4-3.8% increase for both groups Post menopausal 1.8-2% increase for both groups Adherence: Premenopausal: 58%, Postmenopausal: 63%	Effect on BMD only for premenopausal. AIs were a strong predictor of bone loss Improvements in physical performance: Figure-8 running, compared to control. 2km walk time improved in premenopausal trainees more than control. No difference for post menopausal. Change in control group activity was large and positive. No dose-response seen for exercise and bone

RCT: Randomised controlled trial; Ca: Cancer; Sx: Surgery, CTx: Chemotherapy; RTx: Radiotherapy; AIs: Aromatase Inhibitors; Rx: Treatment
HR: Heart rate; AET: Aerobic exercise training; RET: Resistance exercise training; 1-RM: 1 repetition max; 8-RM: 8 repetition max; Wt: Weight; Ht: Height; LBM: Lean body mass; BMD: Bone mineral density; DEXA: Dual energy X-ray absorptiometry; QOL: Quality of life; HRR: Heart rate reserve; RPE: Relative perceived exertion; FFQ: Food frequency questionnaire; PA: Physical activity; CV: Cardiovascular; Time since Dx: time since diagnosis; IGF: Insulin like growth factor; HOMA: Homeostasis Model Assessment; DHQ: Diet History Questionnaire; PAR-Q: Physical Activity Readiness Questionnaire; CARES-SF: Cancer rehabilitation evaluation system – short form; T2DM: Type 2 Diabetes Mellitus; NCI F&V: National Cancer Institute Fruit and vegetable screen
INT: Intervention group; CON: Control group; ITT: Intention to treat; mths: Months;

Resistance exercise training protocols during chemotherapy

One high quality controlled trial, has assessed the effect of resistance training on LBM changes during chemotherapy. Courneya et al reported a significant increase in LBM with no effect on body fat%(Courneya et al. 2007). Those with later stage disease experienced significantly greater gains in LBM compared to control or aerobic exercise, while decreases in body fat% was on par with aerobic exercise (Courneya et al. 2007). Of note, those who completed the resistance training protocol were also more likely to complete their chemotherapy regimen. This is an important consideration for those undergoing treatment, in that exercise may improve primary treatment, not just improve outcomes in relation to metabolic health and physical function.

Combined resistance and aerobic exercise training protocols during chemotherapy

Combined aerobic and resistance training may reduce body fat% when compared to usual care(Battaglini et al. 2007, Winningham et al. 1989), and may increase LBM simultaneously (Battaglini et al. 2007, Winningham et al. 1989). While findings are encouraging and consistent, caution should be exercised in interpreting these results as both studies utilised skinfolds, which may have reduced precision particularly in overweight/obese women (Kuczmarski, Fanelli, and Koch 1987)

A number of limitations are found within the exercise intervention literature. Exercise training has not been shown to reduce body weight in those diagnosed with breast cancer. It is considered that 300 minutes/week of moderate intensity exercise is required for significant weight loss without dietary intervention (Jakicic and Otto 2005). So far, no studies performed in breast cancer populations have aimed for more than 270 minutes of weekly exercise, which may partly explain this lack of body weight change. All exercise interventions during chemotherapy failed to control for dietary energy intake. On the other hand, previous observational studies have found no consistent relationships between dietary energy intake and body composition changes (Demark-Wahnefried et al. 2001, Harvie et al. 2004, Goodwin et al. 1999). All exercise interventions ranged from 90 – 150min of prescribed exercise each week, although significant findings were only found in those programs that included objectively measured and supervised exercise (Battaglini et al. 2007, Winningham et al. 1989, Courneya et al. 2007) as opposed to home-based exercise (DeNysschen et al. 2011). Considering these limitations, interventions that combine nutrition and exercise prescription should be conducted to better control for energy intake. Furthermore, clinicians should be aware that both supervision and objective monitoring of exercise interventions are important to best achieve desired outcomes of body composition change.

Current findings from one high quality study indicate that in those completing treatment, aerobic exercise training alone may be effective in reducing body fat%, while resistance training may be effective in increasing LBM, and importantly, these changes can occur during chemotherapy (Courneya et al. 2007). Combined aerobic and resistance training may confer benefits for both LBM and body fat% simultaneously (Battaglini et al. 2007, Winningham et al. 1989).
Of interest, the greatest relative improvements in body composition during treatment have been found in those with later stage disease (Stage IIB/IIA) (Courneya et al. 2007). These preliminary findings indicate that further investigation in this area is warranted (Irwin et al. 2003).

Exercise interventions and body composition after treatment

Of the eight studies that assessed LBM changes, four studies noted a significant increase for the individuals within exercising groups (Irwin, Alvarez-Reeves, et al. 2009, Schmitz, Ahmed, et al. 2005, Herrero et al. 2006, Fernández-Lao et al. 2013), while one additional study indicated a benefit only for exercisers being treated with aromatase inhibitors (AIs) (Winters-Stone et al. 2011). Three studies indicated no significant difference for the exercisers compared to control (Matthews et al. 2007, Schmitz et al. 2009, Saarto et al. 2011). Controlled exercise trials after adjuvant treatment were typically designed using conventional aerobic and resistance training, however Tai Chi(Mustian et al. 2004) and water-based resistance training protocols (Fernández-Lao et al. 2013) were also conducted.

Of the 17 controlled trials published, exercisers experienced significantly greater decreases in body fat% or skinfold measures in six studies (Fernández-Lao et al. 2013, Schmitz, Ahmed, et al. 2005, Irwin, Alvarez-Reeves, et al. 2009, Herrero et al. 2006, Burnham and Wilcox 2002, Courneya, Friedenreich, et al. 2003). Two studies indicated the same but statistically non-significant trend ($p \leq 0.15$) (Matthews et al. 2007, Courneya, Mackey, et al. 2003) (only studies reporting LBM are summarized in Table 2.1)**.** Compared to non-exercise groups, exercisers experienced a greater decrease in waist girth in one study (Guinan et al. 2013), better maintenance, i.e. stability instead of loss, in one study (Fernández-Lao et al. 2013), and no change in two studies (Irwin, Alvarez-Reeves, et al. 2009, Schmitz, Ahmed, et al. 2005).

Overall, individuals who participated in supervised exercise interventions were more likely to experience a significant increase in LBM compared to control groups. All studies reporting a significant change in LBM and five of the six studies that reported a significant change in body fat% utilised supervised exercise protocols (Schmitz, Ahmed, et al. 2005, Fernández-Lao et al. 2013, Irwin, Alvarez-Reeves, et al. 2009, Herrero et al. 2006, Burnham and Wilcox 2002). Only one home-based protocol reported a significant reduction in body fat%(Courneya, Friedenreich, et

al. 2003), however a number of studies prescribing supervised exercise reported no differences between groups (Daley et al. 2004, Guinan et al. 2013, Mustian et al. 2004, Ligibel et al. 2008, Winters-Stone et al. 2011, Saarto et al. 2011, Schmitz et al. 2009, Courneya, Mackey, et al. 2003). Menopausal status, length of intervention, nor sample size was seen to influence changes in body composition.

Aerobic training and body composition after treatment

Results from aerobic training studies alone have been mixed in regard to body composition change. Typically, protocols have prescribed three to five sessions per week, with duration ranging from 30 to 45 minutes and intensity from 55 to 75% of estimated HRmax. Of the three studies that used a high quality body composition measure (DEXA), Irwin et al (Irwin, Alvarez-Reeves, et al. 2009) was the only study to be employ a supervised (three supervised, two unsupervised each week) and objectively monitored exercise protocol (Heart Rate (HR) monitors). Furthermore, it was the only study to report significant increase in LBM and decrease in body fat% in favour of the exercise group. However, no significant change was seen in waist girth (mean differences: LBM +0.69kg, BF%: -3.0%)(Irwin, Alvarez-Reeves, et al. 2009). Matthews et al (Matthews et al. 2007) and Denysschen et al (DeNysschen et al. 2011) both utilised subjective prescription of exercise intensity (Borg Scale), unsupervised aerobic exercise programs, and did not report statistically significant differences between groups for LBM or body fat%. Both of these interventions set objective targets for volume of exercise to perform that were measured through self-report, and both supported participants through phone counselling. In studies that have objectively prescribed and monitored aerobic exercise intensity with heart rate monitors or tailored treadmill workloads, exercisers have experienced significantly greater reductions in body fat%, albeit using lower quality measures of body composition (BIA and skinfolds)(Courneya, Friedenreich, et al. 2003, Irwin, Alvarez-Reeves, et al. 2009, Burnham and Wilcox 2002). This may indicate that exercise prescription based on subjectivity (Borg scale) and performed at home leads to a lower level (volume and intensity) of activity being performed, and thus a lesser effect when compared to non-exercising control groups.

Further sub-group analysis by Irwin et al (2009) indicated that compared to non-exercise groups, those younger than 56yrs, or with a BMI of more than 30kg/m^2 experienced significantly greater increases in LBM (Irwin, Varma, et al. 2009). In contrast, benefits related to body fat% were significantly higher for women who were over 56yrs of age, were non-obese, had later stage disease (II-IIIA versus 0-I); or were taking any endocrine therapy versus none (Irwin, Alvarez-Reeves, et al. 2009). The authors proposed that younger individuals are more likely to experience chemotherapy-induced menopause and lose more LBM post-diagnosis. Due to this greater LBM loss experienced by these women, an intervention that reverses or even halts the changes will make the loss in a

control group more pronounced. Thus those at the highest risk of adverse change may receive a greater net benefit from exercise than women who experienced natural menopause and are not as prone to the LBM loss (Irwin, Alvarez-Reeves, et al. 2009).

Resistance training and body composition after treatment

Resistance training alone may increase LBM and decrease body fat%. Of the three well-designed RCTs (Schmitz, Ahmed, et al. 2005, Schmitz et al. 2009, Winters-Stone et al. 2011), two noted a significant benefit for the exercise group (Schmitz, Ahmed, et al. 2005, Winters-Stone et al. 2011), while the other found no difference between groups(Schmitz et al. 2009).

Winters-Stone et al (Winters-Stone et al. 2011) did not find whole group differences between exercise and control (DEXA - mean difference: +0.26%, p=0.91). However, exercisers taking AIs experienced statistically significant increases in LBM, while those in the control group did not experience any change in LBM regardless of treatment with AIs (group x AI on slope of time; t(95) = -2.51, p=0.01) (Winters-Stone et al. 2011). This extends findings that AIs had previously been reported in observational studies as having an independent and anabolic effect on LBM thought to be due to their androgenic properties (van Londen et al. 2011, Montagnani, Gonnelli, et al. 2008, Francini et al. 2006).

The three resistance exercise trials used supervised progressive training twice-weekly, while Winters-Stone et al (2011) also prescribed a jumping program consisting of 20-60 jumps per session, and an additional home theraband strength session once per week. Schmitz et al (2005)(Schmitz, Ahmed, et al. 2005), and Schmitz et al (2009)(Schmitz et al. 2009) both prescribed progressive resistance training, however differences in frequency and intensity existed between protocols. The 2005 study reported a +0.86kg mean difference in change of LBM in favour of the training group (Schmitz, Ahmed, et al. 2005), while no effect was reported in 2009 (Schmitz et al. 2009). Differences in primary outcome, population selection and training regimes may explain these inconsistencies. The 2009 study was designed to assess safety of resistance training for women with lymphoedema (Schmitz et al. 2009) as opposed to the 2005 study, which aimed to assess general safety and efficacy of resistance training in body composition change (Schmitz, Ahmed, et al. 2005). The 2005 study also recruited participants who were more recently diagnosed with breast cancer (mean of 1.8 years, versus 6.5 years post-diagnosis).

Typically, the greatest body composition changes occur in the first six to 12 months following treatment and may continue for three to four years, albeit more slowly (Makari-Judson, Judson, and Mertens 2007, Harvie 2010). Resistance training sooner after treatment may therefore create a greater disparity in anabolic status when compared to an extended time post-treatment. In addition, the 2009 study recruited only those with past or current clinically diagnosed lymphoedema, while

these individuals were excluded from the 2005 trial. Participants with lymphoedema may have been more cautious with their weight progression, which may have reduced their potential gains. Supporting this, the 2005 study noted a 63% improvement in 1-RM bench press, compared to a 29% improvement in the 2009 study.

Three supervised sessions per week may have elicited a greater change than twice weekly(Schmitz, Ahmed, et al. 2005, Schmitz et al. 2009) or twice weekly plus one unsupervised session (Winters-Stone et al. 2011) as the study during chemotherapy by Courneya et al (2007) noted significant LBM changes for the resistance exercise group at this frequency (Courneya et al. 2007). Similarly, studies from healthy populations would indicate that LBM gains may increase with a higher weekly dose of resistance exercise (Rhea et al. 2003), while more recent literature indicates that lifting to temporary failure is an important key to maximal muscle protein synthesis (Breen et al 2011).

Further research needs to investigate the mediating effects of later stage disease, earlier intervention (immediately post treatment) and changes in function as a result of training, however resistance training can be considered safe for all breast cancer survivors with or without lymphoedema. One trial assessing safety of exercise reported a 10% injury rate related to the intervention itself, with only one injury being severe enough to stop participation in the intervention (Schmitz et al 2005). Of note, lower back injuries were the most common accounting for 50% of all intervention related injuries reported. Schmitz et al (2009) noted a significantly lower rate of exacerbations of lymphedema was reported as a result of resistance training when compared to the control group. Finally, adherence over 12 months of both trials was reported to be ~80%.

Combined aerobic and resistance training and body composition after treatment

Exercise interventions that have combined both resistance and exercise training have had positive effects on LBM, however the data is currently limited by the use of lower quality measures of body composition.

The two studies reporting LBM change noted a significant increase in LBM after land-based training (Herrero et al. 2006, Fernández-Lao et al. 2013). Herrero et al reported a significant increase in LBM in favour of the exercise group (mean difference: +1.0kg 95%CI: 0.25 to 1.86) after eight weeks of training measured by skinfold assessment (Herrero et al. 2006). In addition, Fernandez-Lao (2013) reported a significant group x time interaction for the land-based resistance and aerobic training over water-based training and control using BIA. (p=0.008) (Fernández-Lao et al. 2013). Both studies prescribed combined training three times per week, with a total weekly duration ranging from 180(Fernández-Lao et al. 2013) to 270mins (Herrero et al. 2006) per week, both employed progressive overload for both resistance and aerobic protocols and supervised all sessions in gym environments using free and body weights. Markers of strength and

cardiorespiratory fitness were reported by Herrero et al, and compared to non-exercising controls, the exercisers experienced greater improvements in lower body strength, power and VO_{2max} (Herrero et al. 2006). However, Herrero et al (2006) had a small sample size (N=16) and ideally larger trials are needed for more conclusive data.

Body fat% decreased significantly in two studies (Ligibel et al 2008, Fernández-Lao et al. 2013), while one reported waist girth maintenance in the exercise groups compared to a significant increase in the non-exercise control group (Fernández-Lao et al. 2013). In the second study, Ligibel et al (2008) found that 200 minutes of combined training resulted in a decrease in hip girth for the exercise group, however no change was reported for body fat%.

Functional change after exercise training

Despite there being a less consistent effect of exercise training on body composition change, exercise trials have consistently shown significant increases physical function. Resistance training programs have reported increases in 1-Repetition max (1-RM) strength from 30-60% after 12 months of training (Schmitz et al 2005, 2009). In addition, aerobic exercise after treatment has resulted in 18.6% improvements in VO2max (Burnham et al 2002) compared to no change in the control group. However, during chemotherapy, aerobic training maintained VO2max, while control and resistance training groups experienced a decline over the course of treatment. (Courneya et al 2007)

LBM function and strength has been shown to be more predictive than absolute values of LBM or fat mass (Ruiz et al 2008, Newman et al 2006). Thus, future research aiming to assess the effects of an intervention on body composition should also assess the effect on physical function.

Conclusions for controlled exercise trials conducted after treatment

Compared to resistance training alone, supervised and/or objectively monitored aerobic exercise training is indicated as the preferred protocol to reduce body fat% and measures of girth. As expected, compared to aerobic exercise training, resistance training is more likely to increase LBM. Thus, combined resistance and aerobic exercise training may simultaneously improve LBM and body fat%, and be the preferred prescription. However future studies should prescribe training three times per week in order to determine benefit of a given protocol.

A major limitation in the literature stems from the lack of premenopausal participants included in completed studies. All studies conducted so far have included primarily postmenopausal populations. Considering premenopausal women experience adverse body composition changes of larger magnitude than postmenopausal women following treatment (Vance et al. 2011), future

studies should include this population to make the information more generalisable to all breast cancer survivors.

Aiming for LBM maintenance or growth with a reduction in body fat% and/or waist girth is a clinically relevant goal for breast cancer survivors participating in an exercise program alone without specific dietary prescription.

Summary of the effects of exercise training on measures of body composition

During chemotherapy and after treatment completion, progressive resistance training with a frequency of at least twice per week is likely to elicit the greatest LBM gains, while aerobic and combined resistance and aerobic training protocols are more likely to positively affect body fat% and waist girth compared to non-exercising control groups.

The greatest difference in LBM change reported between groups is 1kg (Herrero et al. 2006). Reasons for this low magnitude change may be due to a number of reasons. Firstly, breast cancer survivors are predisposed to LBM loss (Harvie 2010), thus maintenance or small increments may be considered a clinically significant outcome. Secondly, dietary intake has been inadequately assessed in the current studies. The lack of focus on supportive anabolic nutrients in breast cancer trials may have confounded changes in LBM or general body composition. Finally, volume of exercise protocols may have been too low to achieve a greater magnitude of change, i.e. ≥ 3 sessions/week may have produced more consistent results (Rhea et al. 2003). However, lower volumes of resistance exercise (120min/week of resistance training)(Schmitz, Ahmed, et al. 2005) have been shown to elicit desirable body composition changes, i.e. a decrease in body fat% and increase in LBM.

In breast cancer survivors, body fat% has been most effectively reduced by supervised and/or objectively monitored aerobic exercise both during and after chemotherapy. Resistance training alone and combined aerobic and resistance training may increase LBM and reduce body fat% simultaneously, which is considered the optimal outcome for these individuals.

Preliminary findings have shown that exercise training may positively affect treatment with AIs as they modify hormonal profiles to be more androgenic (Winters-Stone et al. 2011). Greater reductions in body fat% as a result of exercise training may occur in those: with later stage both (during and after treatment)(Courneya et al. 2007, Irwin, Alvarez-Reeves, et al. 2009); older than 56yrs; non-obese; and those on endocrine therapy(Irwin, Alvarez-Reeves, et al. 2009)..

Limitations of the current literature are the lack of premenopausal women included in trials, and for those with differing disease stages and ages. In addition, studies should be focused both during and

immediately after treatment as this may be the time where most effect can be created (Makari-Judson, Judson, and Mertens 2007).

Finally, and more practically, women are more likely to have greater relative gains in strength than increases in LBM cross-sectional area (Hubal et al. 2005). Cardiorespiratory and muscle function (strength/endurance) can increase by up to 18-20% and 60%, respectively, post intervention. Improvements in cardio-respiratory fitness, independent of body weight change, are associated with better quality of life (Ingram, Courneya, and Kingston 2006) in breast cancer survivors, and reduced risk of cardiovascular disease and diabetes in non-breast cancer populations (Arsenault et al. 2009, Gau et al. 2010). Increased strength is associated with greater quality of life in breast (Herrero et al. 2006) and non-breast cancer survivor populations (Dale et al. 2013, Heesch et al. 2012, Beniamini et al. 1997).

Therefore, the key outcome measure considerations when determining the efficacy of an exercise training intervention should include:

1. Body composition change: LBM maintenance or growth with moderate decreases of body fat% and/or waist girth.
2. Functional change: improvements in strength and or cardiorespiratory fitness.

2.2.2 Body composition changes after dietary intervention alone

Numerous dietary interventions exist in breast cancer populations. Similar to exercise, a vast majority of the interventions have been focused on or include a measure of body weight change. Observational studies have indicated an inconsistent relationship between body weight and fat mass gains and dietary energy intake (Demark-Wahnefried, 2001, Harvie et al 2004). In one study, weight increase was reported in the presence of a dietary energy deficit. Interventions focusing on energy restriction or diet quality have revealed more predictable findings in regard to weight, with potentially adverse effects on body composition.

Dietary energy restriction results in total body weight loss, however it may be at the expense of LBM (Thompson et al 2010). Interventions focused on healthy changes to improve diet quality without energy restriction often achieve body weight losses of 0.5kg to 3kg (Chlebowski et al 2006, Saquib et al 2008, Villarini et al 2012, Hebert et al 2001), however LBM has not been reported in these trials. An intervention focusing on anabolic nutrients is required to better investigate LBM during weight loss in breast cancer populations. Nutrients with anabolic properties have not yet been used in a population of women who have been treated for breast cancer, and present as an important consideration in diet only trials.

In all, 10 controlled trials that have assessed an element of prescribed dietary alteration and reported at least one outcome of body composition (Table 2.2). Six of these studies aimed for body weight

loss through dietary energy restriction (N=306)(Shaw, Mortimer, and Judd 2007a, Shaw, Mortimer, and Judd 2007b, Djuric et al. 2002, de Waard et al. 1993, Thomson et al. 2010, Flynn and Reinert 2010), and four studies, producing multiple articles, have evaluated the effect of micronutrient or food quality alteration on without prescribing energy restriction (N=5327)(Chlebowski et al. 2006, Saquib et al. 2008, Thomson et al. 2005, Villarini et al. 2012). Generally, studies performed after 2003 were of high quality (Thomson et al. 2005, Chlebowski et al. 2006, Thomson et al. 2010, Shaw, Mortimer, and Judd 2007a, Shaw, Mortimer, and Judd 2007b, Villarini et al. 2012), and one was ranked as neutral quality (Flynn and Reinert 2010). Earlier studies were limited by poor demographic/disease selection or description (de Waard et al. 1993, Hebert et al. 2001), or lack of equal groups at baseline (Djuric et al. 2002).

Body mass was reported in all 10 studies, body fat% as measured by DEXA or BIA was reported in six studies(Shaw, Mortimer, and Judd 2007b, Thomson et al. 2010, Thomson et al. 2005, Jen et al. 2004, Flynn and Reinert 2010, Villarini et al. 2012), skin fold thickness was reported in two studies (Shaw, Mortimer, and Judd 2007a, Villarini et al. 2012). LBM was reported in four studies, one using a high quality measure (DEXA)(Thomson et al. 2010), and three others used BIA (Thomson et al. 2005, Villarini et al. 2012, Flynn and Reinert 2010).
Eight of ten studies reported more postmenopausal participants (range: 64% to 100%), in addition, participants had completed treatment for breast cancer more than one year before trial entry in all but three studies (Chlebowski et al. 2006, Hebert et al. 2001, Villarini et al. 2012). Considering both premenopausal status (Goodwin et al. 1999, Harvie et al. 2004) and a shorter duration since completing therapy (Makari-Judson, Judson, and Mertens 2007) are associated with larger changes in body composition, studies are currently lacking to describe changes for these populations.

TABLE 2.2 CONTROLLED TRIALS EVALUATING DIETARY INTERVENTIONS ON BODY COMPOSITION IN BREAST CANCER SURVIVORS

Author, Year, Design, Country, Quality, Primary outcome	**Population**	**Intervention**	**Body composition and other outcomes**	**Comments**
Interventions using dietary energy restriction (N=262)				
de Ward, 1993 RCT 2 arm Netherlands (Ned) & Poland (Pol) Multicentre NHMRC: II Quality: - Body composition	N=102 (initial) Pol: n=48; Ned: n=54 Intervention: n=59 Control: n=43 Age: 50-69yrs Postmeno: 100%, BMI >27kg/m^2 Stage: Not defined	Duration: 3 years follow up Follow up: 3yrs Ned, 1 yr Pol INT: Dietitian delivered (unspecified no. of sessions); 1500kCal intake, with further reduction to 1000kCal if weight loss not being achieved. CON: Usual care. Had access to weight loss advice on request. Measures: Body weight	Results after 1 year Significant ↓ in body weight Overall: INT: -6kg, CON: +1kg, P<0.001 Ned: Weight loss at 3 years was correlated to weight loss after 1 year, r=0.91.	Weight loss at 1 year reflected loss at 3 years No details of demographics. Limited information re: intervention intensity. No body composition breakdown 60% follow up at 3 years
Djuric, 2002, Jen, 2004 USA RCT 4-arm Quality: O Body composition	N=48; Age: 52.1yrs (8.4); Weight: 94.5 (13 SD); Postmeno: 75%; CTx: 63% Current tamoxifen: 63%; Diagnosed within last 4 yrs; co-morbidities. Differences in metabolic biomarkers, similar BMI and weight.	Duration: 12 mths WW: Weight watchers, DC: Dietary counselling-2100-4200kJ deficit + 30min PA/day WW+DC: Weight watchers and dietary counselling + recommendation to increase exercise Contact: WW – weekly; DC: Weekly for 12 weeks, bi-weekly for 12 weeks CON : Healthy Eating for Cancer Measures: BIA: Tetra polar Wt, Ht, 3-Day food records FACT-An	Significant ↓ in body weight (kg) WW: -2.7, DC: -8, WW+DC: -9.5, CON: +1.1; WW+DC>DC>WW>CON, All significant to P<0.05. Significant ↓ body fat% WW: -0.99, DC: -3.17, WW+DC: -3.65, CON: +0.23; WW+DC=DC>WW=CON WW+DC only group with significant within group change. Greater attendance associated with greater loss; Caloric intake did not correlate to results; Benefits for weight loss and improvements in TChol, HDL; WW+DC ↓ leptin	Study showing Wt loss achievable and will come with good changes in CVD markers Time intensive education to achieve results. Body fat% change indicate significant loss of LBM by deduction PA measure lacking sensitivity

Shaw, 2007 RCT, 2-arm UK Quality: + NHMRC: II Arm volume	N=21; Age:60yrs (median); BMI: 32 (mean); Time since Dx: >1yr; BMI >25 No differences between groups	Duration: 12 weeks INT: Dietitian delivered. 4200kJ deficit from habitual diet, all diets >4200kJ/d. ↓ refined CHO and ↓ high fat foods. CON: Healthy eating guidelines Exercise not discussed Contact: Monthly Measures: skinfolds (4-site), Wt, Ht, 7-day food diary, Arm volume, Waist, Hip Bloods: TG, TChol, Carotenoids, Insulin 3-day food diaries	Significant ↓ in body weight (kg) INT: -3.3, CON: 0.0, P=0.02 No significant change in skinfolds (mm) INT: -5, CON: 2.5, P=0.426 Arm volume (ml)- affected arm: -350 vs. -11, P=0.003 Correlation of weight loss and change in arm volume: R=0.513, P=0.017	Weight reduction was effective, without a significant reduction in skinfolds. No record of LBM change. Weight reduction was correlated to reductions in arm volume. No record of PA or encouragement Generalisable to without lymphoedema? Long enough for an effect
Shaw, 2007 RCT, 3-arm UK Quality: + NHMRC: II Arm volume	N=51 Age: 69yrs:Lymphoedema: 100%; Diff in arm volume: 44% BMI: 29 (mean) Time since Rx: >12mth, +/- hormonal Rx	Duration: 6 months INT-Weight Reduction (WR): Dietitian delivered. 4200-5050kJ energy deficit INT-Low Fat (LF): <20% of energy from fat CON: Continue eating habits Exercise not discussed or measured Contact: Unknown Measures: skinfolds (4-site), Ht, Wt, 7-day food diary, Girth of swollen limb & Perometry	Significant ↓ in body weight (kg) INT-WR: -4, INT-LF: -2.6, CON: -0.6 Diff between groups, P=0.006 Significant ↓ in body fat% INT-WR: -2.8, INT-LF: -1.4, CON: -0.4, Diff between groups, P=0.017 No significant difference in change of excess arm volume Weight reduction had a significant effect on reduction of swollen arm (irrespective of dietary group) Spearman rank 0.423, P=0.002	Skinfolds in overweight/obese. Weight loss successful for ↓ in refined CHO and high fat foods. Adherence to diet: 42% and 59% for weight loss and low fat. All but 1 lost weight in weight loss group. Intention to treat analysis.

Interventions aiming for weight stability and macro/micronutrient alteration (N=5327)				
Chlebowski, 2006 RCT 2-arm USA Quality: + NHMRC: II BrCa survival	N=2437; INT: 975, CON: 1462. Data for BMI @ Baseline: N=2381 Yr 1: N=1985; Yr 3: N=1581; Yr 5: N=847 Final; White: 84.75, time since Dx: <1yr, Menopausal: N/A; Stage I-IIIA; CTx: 52.4%; T2DM: 5%; BMI>26: 54.1%	Follow up 5 years INT: Reduce fat to 15%. Individual fat gram goal 8 x 1hr Dietitian sessions, plus f/up every 3 months. Isoenergetic intake with less fat CON: General healthy eating, nutritional adequacy of vitamins and minerals only Measures: Wt, Ht	Significant ↓ in weight for INT Mean difference b/w INT & CON: Yr 1: -2.3kg, p<0.05; Yr 3: -1.8kg, P<0.05; Yr 5: -2.7kg, P<0.05. INT: ↑ Relapse free survival, 24%, P<0.05 ↑ Recurrence free survival, 29%, P<0.05 ↑ Disease free survival, 19%. P<0.05 No difference in survival	Weight loss not an aim, saw significant reduction in weight at 1, 3, 5yrs. Significant attrition. Results strongest for ER-ve status
WHEL Saquib, 2008 Thomson, 2005 RCT 2-arm Multicentre USA Quality: + NHMRC: II BrCa Survival	N=2718 53.4yrs (26-74) BMI: 27.3kg/m^2 (57% OW); Race: 85% white; Stage III: 4,2% *Thomson (subset)* N=52 – means of groups Age: 55.9 & 52.3yrs, Race: 100 % 90% white; Menopause: Pre: 9.5, Peri: 14.3, Post: 76.2; time since Dx: <4yrs No sig diff b/w groups	4 yrs ongoing INT: 5 vegetable serves, 450ml vegetable juice, 3 fruit serves, 30g fibre, and 15-20% energy from fat. Telephone counselling, cooking classes and newsletters. CON: print materials with general healthy eating tips (Source: US Dept Agriculture) Common Measures: Wt, Dietary: 4 x 24hr dietary recalls, PAQ Thomson: BIA, Waist, Hip (6, 12, 48mths)	Significant ↓ body weight (kg) at 1-yr 1-year: INT: -0.05, CON: 0.71, p<0.0001 4-yr: INT: +1.77, CON: +1.43, NS Thomson (N=52) Trend for ↓ body weight @ 48mths (RMANOVA) INT: +0.73kg, CON: +1.93kg, P=0.1 No sig diff among time points, P=0.32 Significantly lower body fat% over time (baseline, 6mth, 12mth) INT: Bline: 31.6%, 6m: -1.5, 12m: +1.04 CON: Bline: 31.1%, 6m: -0.1, 12m: +2.27 Sig diff among time points, P=0.04 Significantly ↓ LBM (kg) for INT group Mean change: INT: -0.62kg, CON: +0.64kg P=0.048 No difference in body fat% or waist girth change at 48 months between groups	Body fat% was lower in INT after 6mth, but not after 48mths. Mean change in LBM over 48mths was reduced in INT group. Changes are within limits for BIA. Different mean weight changes compared to larger population. Total energy intake did not decrease, despite energy density decreasing

Hebert, 2001 RCT 3 arm Quality: O NHMRC: II Body composition	. N=172, N=157 final; Postmeno: 63.1%, Time since Dx: <12mth: 61.2%. Stage I-II 20-65yrs; CTx: Ever: 61.2% (During: 14%) No difference between groups	Duration: 4mth, 12mth f/up SRC: Mindfulness stress reduction. NEP: 2 individuals + 14 x 150 minute group sessions + 1 full-day (5.5 hr) workshop. Diet contained: 20% fat, high fibre - education and support. Weight loss not a priority. CON: given free choice Measures: 7Day diet recall-FFQ, Beck Anxiety Index, BDI, Self-esteem, Symptom checklist	.Significant ↓ in body weight @ 4 mth NEP: -2.4kg, SRC: 0kg,CON: +0.2kg, P<0.05 NEP vs SRC & CON No significant change @ 12mth NEP: +0.1kg, SRC: +0.4kg, +0.5kg, P>0.05 Those with higher expectation of NEP outcomes performed better than those with low outcomes. Class attendance did not affect weight loss	Healthy eating with no weight loss goal reduced weight over 4 mths but not 12 months. Expectation of randomisation did not affect outcomes
Villarini, 2012 RCT 2 arm Milan, Italy NHMRC: II Quality: +ve Body composition ..	Invasive BrCa, Scheduled for CTx, no metastases, Milan, n=96 INT: Age: 52.7 (10.8), Ht: 1.61 (0.07), Wt: 63.8 (11.8); BMI: 24.7 (4.5); BP: 128, DBP: 82.4 CON: Age: **48.4** , Ht: 1.63, Wt: 64.7, BMI: 24.7, SBP: 131, DBP: 85.6	Start to finish of adjuvant chemotherapy INT: Cooking classes and common meals twice per week Macrobiotic diet – rice, millet, spelt, barley, corn, pulses, vegetables, fermented soy, tofu, seaweeds, fish and sugar/fat free desserts, fruit, wine, cheese, yoghurt, eggs and meat CON: Given baseline education from INT group, and information on reducing side effects of cancer Measures: Time point (TP) 1-Bline, TP2-end of 1st cycle and TP3-end of CTx Waist and hip, weight BIA, skinfolds (4-site); 39-item food frequency	Significant decrease in weight for INT group compared to control **Weight** TP1 to TP2: INT: -2.7kg, CON: -1.4kg, p=0.06 TP1 to TP3: INT: -2.9kg, CON: -0.1kg, p=0.00 **LBM** TP1 to TP2: INT: -0.8kg, CON: -0.7kg, p=0.45 TP1 to TP3: INT: -0.7kg, CON: +0.1kg, p=0.01 **Fat mass** TP1 to TP2: INT: -2.6kg, CON: -1.4kg, p=0.32 TP1 to TP3: INT: -2.3kg, CON: -0.7kg, p=0.03 **Waist:** TP1 to TP2: INT: -2.7cm, CON: -1.5cm, p=0.05 TP1 to TP3: INT: -3cm, CON: -0.1cm, p=0.01 **Hip:** TP1 to TP2: INT: -1.4cm, CON: -1.5cm, p=0.23 TP1 to TP3: INT: -1.7cm, CON: +0.1cm, p=0.03	Healthy diet information to both groups may have influenced results, as CON decreased weight and fat mass. Extremely high retention rates.Weight gain is minimal on anthracyclines, but most notable on CMF, where the weight gain was only seen in non-dietary arm. Some LBM lost, ~30% of total weight lost.

Flynn, 2010 Cross-over RCT 2-arm USA NHMRC: II Quality: O Different effects of diet	N=44 (20 completed 6 months); 59.2yrs (52-73); BMI: 27.9±2.8kg/m^2, Body fat%: 41.6±4.6, LBM: 58.6±5.1% No data given on other demographic categories. No ITT	8 week interventions for each diet – order randomised. Diet 1: Plant based Olive oil (PBOO) 1500cal; unlimited veg; 3 tbsp of olive oil/day, 3 servings of fruit, 6oz/week poultry, 8oz/week seafood. Mainly vegetarian meals. National Cancer Institute (NCI) diet: 25-50g fat; unlimited F&V minimum (5 serves/day); 7oz of meat, Canola oil provided for cooking. Measures: Ht, Wt, BIA, waist hip girth.Bloods: TG, Chol, Carotenoids, Insulin, 3-d food diary	Significant ↓ body weight (kg) for PBOO vs NCI: PBOO: -3.6kg vs -2.7kg, p=0.05, No significant difference in %LBM change PBOO: +1.9% vs NCI: +1.1%, p=0.12 No significant difference in body fat% change: PBOO: -1.9% vs NCI: 1.4%, p=0.28 No significant change in waist girth: PBOO: -3.4 vs NCI: -2.6, p=0.25	PBOO diet was more efficacious for weight loss than a standard low fat diet despite energy intake being greater for the PBOO diet. The PBOO was more palatable, more likely to be chosen for the 6 month follow up. No absolute value of LBM was given making body composition change difficult to interpret Limited demographical information given.

Wt: Weight; Ht: Height; BMI: Body mass index; LBM: Lean body mass; aLBM: appendicular LBM; OW: Overweight; BIA: Bioelectrical impedance; Postmeno: Postmenopausal; CRP: C-reactive protein; HOMA: Homeostasis Assessment Model; TChol: Total Cholesterol; PA: Physical activity; PAQ: Physical activity questionnaire; RER: Respiratory Exchange Ratio; CHO: Carbohydrates; FACT-An: Functional Assessment of Cancer Therapy-Anaemia subscale; CTx: Chemotherapy; PBOO: Plant based olive oil diet; TP: Time point; INT: intervention group; CON: Control group; BrCa: Breast Cancer

Dietary interventions evaluating the effects of dietary energy restriction on body composition

All six studies that prescribed a dietary energy restriction have reported statistically and clinically significant reductions in body weight for intervention groups. Significant reductions in body fat% were generally consistent within studies when they were reported. Prescribed daily energy deficits ranged from 2100kJ to 5050kJ in five studies (Shaw, Mortimer, and Judd 2007a, Shaw, Mortimer, and Judd 2007b, Djuric et al. 2002, de Waard et al. 1993, Thomson et al. 2010), while one delivered a standard 6250kJ (1500Cal) per day (Flynn and Reinert 2010). To date, no trials have targeted specific nutrients that may preserve LBM, the major focus of published studies thus far have been on total body weight loss.

After a body weight loss of ~6kg (5.9kg and 6.3kg for the two intervention groups, respectively), LBM was significantly reduced after 6 months energy restriction in one high quality study (Thomson et al. 2010), while a neutral quality study reported no change in LBM after 8 weeks of energy restriction, however no data was shown (Flynn and Reinert 2010). The high quality study by Thomson et al (2010) reported that prevalence of myopenia, defined by an appendicular LBM (aLBM) measurement of $<5.67 kg/m^2$, increased from 8% to 18% for the entire study population (both groups on energy restricted diets) (Thomson et al. 2010). Considering the predisposition for loss of LBM in this population, exacerbation of this loss is not considered an optimal result. In a healthy population of postmenopausal women, higher dietary protein was correlated with reduced losses of LBM and appendicular (aLBM) even after controlling for exercise (Easter et al. 2008), however associations between protein intake and LBM change were not reported for this study due to inadequate power (Thomson et al. 2010).

Over the six studies, reductions in body weight ranged from 2.6kg to 9.5kg, and were achieved over a range of 8 to 52 weeks. Longer studies with follow up of 6 months or more were those that found the greatest losses in body weight >6kg (Thomson et al. 2010, de Waard et al. 1993, Djuric et al. 2002). Individuals within intervention groups were typically contacted weekly for the first 8 to 12 weeks, with a decrease in contact over time. Regularity of contact had little influence on magnitude of weight loss, however Jen et al (2004) identified that participants exposed to dietitian-led dietary education and a Weight Watchers program, those participants lost significantly more weight than the dietitian-only group, which in turn was better than the weight watchers program alone (Jen et al. 2004). Considering Weight Watchers was continued weekly for the duration of the study, and the dietitian-led education was reduced after the first 12 weeks, there may be an advantage to more regular contact throughout the dietary change process.

Two studies compared groups exposed to identical energy restrictions that differed in macronutrient profile with differing findings. Thomson et al (2010) reported comparable results for low carbohydrate versus a low fat dietary prescription over 6 months (i.e. 5.9kg and 6.3kg, respectively) (Thomson et al. 2010). Flynn et al (2010) reported a significantly greater reduction in body weight for those allocated to a Mediterranean dietary pattern that was higher in fat, and lower in protein and carbohydrate diet (PBOO) than one based on the National Cancer Institute guidelines (NCI) (Flynn and Reinert 2010). The greater weight loss in the PBOO group occurred even though dietary recalls indicated a lower daily energy intake in the NCI group (~6100kJ and 4773kJ, respectively). Unstructured subjective data indicated the PBOO diet provided greater palatability, satiety and ease of preparation, meaning greater reductions in body weight may have been a result of better adherence to the diet, rather than macronutrient profile.

As expected in trials that elicited significant reductions in total body weight, a significant decrease in measures of body fat% was found in three studies that reported a measure of adiposity (Thomson et al. 2010, Jen et al. 2004, Shaw, Mortimer, and Judd 2007b), while one reported no difference compared to control (Shaw, Mortimer, and Judd 2007a). Change in waist girth was clinically and statistically significantly reduced in the one trial that reported it (Thomson et al. 2010)
Limitations in studies reporting body composition change after dietary intervention stem from the lack of high quality body composition measures, plus four studies lacked appropriate control for physical activity to determine it's role in body composition change (Shaw, Mortimer, and Judd 2007a, Shaw, Mortimer, and Judd 2007b, Jen et al. 2004, de Waard et al. 1993).

Cardiovascular and metabolic syndrome related outcomes after dietary intervention
While body weight loss may result in LBM loss, biomarkers of cardiovascular and metabolic health were improved. Thompson et al (2010) noted a trend for reduced CRP after weight loss and improvements in HbA1c and triglyceride levels in the low carbohydrate group. Shaw et al (2007a & 2007b)(Shaw, Mortimer, and Judd 2007a, Shaw, Mortimer, and Judd 2007b) noted a correlation for weight loss and reduction in arm volume in a population with lymphoedema, and Jen et al (2004) reported significant improvements in total cholesterol, HDL cholesterol and leptin levels after weight loss (Jen et al. 2004).

Summary of findings from dietary energy restriction interventions

It is evident from current knowledge that body weight loss and reductions in body fat% are achievable in breast cancer survivor populations after dietary energy restriction. This is important, as prospective data has indicated a limited role for dietary intake in treatment related weight gain (Demark-Wahnefried et al. 2002). However, body weight loss may occur at the cost of clinically significant and detrimental losses of LBM (Thomson et al. 2010). Further studies are needed to confirm changes in LBM after diet-induced weight loss, and to determine if the resulting improved metabolic profile in breast cancer survivors is more beneficial than the potential detriment of significant LBM loss. Successful studies have involved frequent contact with a dietitian as individuals or groups, with potentially better results to come from continued frequent contact.

Dietary interventions prescribing a change in dietary pattern without energy restriction

Change in diet quality, or an increase in specific nutrients may be of benefit to mortality and morbidity following treatment for breast cancer. The following dietary interventions have aimed to modify dietary quality as opposed to overall energy consumption. These studies have typically elicited small albeit clinically relevant changes in weight.

Dietary interventions that have focused on altering dietary quality or dietary pattern without energy restriction have resulted in a trend to decrease body weight (0.5kg to -3kg) over time. There are two distinct categories of controlled trials that have prescribed dietary pattern changes without energy restriction: survival studies (Chlebowski et al. 2006, Saquib et al. 2008) and short duration dietary interventions (Hebert et al. 2001, Villarini et al. 2012).

The WINS (N=2437)(Chlebowski et al. 2006), and WHEL (N=2718)(Saquib et al. 2008) studies prescribed a reduced total dietary fat (15% of total energy intake), and reduced total dietary fat (15-20% total energy intake) and increased vegetable intake (>10 serves per day), respectively. Both studies aimed to assess the impact of diet on breast cancer survival. The studies ran for five and four years, respectively, and compared to control groups, both noted a small yet significantly lower body weight (-2.7kg, $p<0.05$ & -0.05kg, $p<0.001$, respectively) for intervention groups after one year follow up. However, only the WINS study reported long-term maintenance of this difference (5-year follow up). The WINS study resulted in a significant reduction in total energy intake, which explained the sustained weight loss for the intervention group (Chlebowski et al. 2006). In contrast, findings from the WHEL study indicated that overall energy density was reduced but the energy content of the greater intake of fruits and vegetables compensated in the intervention group compensated for this and may explain the lack of clinically significant weight change (i.e. -0.05kg) throughout follow up (Saquib et al. 2008).

Thomson et al (2005) reported change in LBM and body fat%, as measured by BIA, in a subset (N=52) of the WHEL study population (Thomson et al. 2005). Compared to control, LBM was seen to significantly decline in the intervention group (+0.64kg vs -0.62kg, p=0.048) over 48 months of follow up. Body fat% gains were attenuated in the intervention compared to control after 6 months, but not 48 months. No such measure was taken in the WINS study. Of note, the WINS study reported benefit in the intervention group for relapse and disease free survival reporting a 65% attrition rate over 5 years. The study indicated a benefit for relapse, recurrence and disease free survival in the intervention group, and this was thought to be a result of the difference in weight change as opposed to the total fat restriction (Chlebowski et al. 2006).

In contrast to the two large trials of long duration, Villarini et al(Villarini et al. 2012) and Hebert et al(Hebert et al. 2001) conducted shorter term dietary interventions (three & four months, respectively) with smaller sample sizes, which differed in dietary prescription. However both reported significant short term reductions in body weight for intervention groups (-2.7kg vs -1.4kg(Villarini et al. 2012); & -2.7kg vs +0.2kg(Hebert et al. 2001). Herbert et al (2001)(Hebert et al. 2001) compared the effect of a dietitian-led nutrition program (NEP), with a mindfulness based stress reduction clinic program and a usual care control after completion of treatment. Villarini et al (2012) prescribed a diet based on Mediterranean and macrobiotic diet principles through chemotherapy with ongoing cooking classes, compared to a baseline cooking class for the control group. Only Villarini et al (Villarini et al. 2012) reported a significant a difference between groups after longer follow up, -2.9kg vs 0.01kg & +0.1 vs +0.5kg, respectively.

Compared to control, Villarini et al (2012) reported a significant loss of LBM (mean difference: -0.8kg, $p<0.01$) and body fat% (mean difference: -1.6%, p=0.03) for the intervention group at the final time point (Villarini et al. 2012).
Change in energy intake reported by Hebert et al (2001) (-280kJ/day) only explained 40% of the total weight loss over 3 months, and did not differ from control groups. On the other hand, while Villarini et al (2012) did not report energy intake, a previous study using the same dietary protocol indicated a reduction in energy intake of 1000kJ per day as a result of the reduced energy density created. This may explain the 2.7kg weight loss over 4 months (Berrino et al. 2001) The small but significant loss of LBM found in intervention groups that reported significant weight loss(Chlebowski et al. 2006) is noteworthy due to the heightened propensity for LBM loss in breast cancer survivors (Thomson et al. 2010).

Dietary interventions that have aimed for dietary pattern change without energy restriction have reported an initial weight loss with mixed results in regards to long term follow up. The magnitude of the weight loss was <3kg amongst all studies at all time points. In contrast, for the two studies that reported LBM change, both reported reductions in LBM as part of the weight lost. However, these findings are limited by the use of BIA as body composition measurement. Considering that LBM loss and cancer related muscle wasting is not a good outcome for women after treatment (Thompson et al 2010), interventions should focus on anabolism of LBM and concurrent fat mass reduction, through either anabolic nutrients like increased and specific protein intake, or the addition of exercise.

Summary of controlled trials evaluating the effects of diet on body composition

LBM losses are common in non-cancer populations during diet-induced weight loss, the specific population of breast cancer survivors may be more adversely affected due to their pre-existing propensity to lose LBM in the absence of overall weight loss (Harvie 2010, Demark-Wahnefried et al. 2001) Compared to control groups, both energy restricted dietary prescription, and diets prescribing specific foods without energy restriction, result in body weight losses of >6kg, and 0.05 to 2.9kg, respectively. Interventions targeting weight loss through energy restriction elicited greater change than those that only looked to alter dietary pattern without restriction.

Interventions aimed at maintaining LBM should be considered in breast cancer populations. Anabolic adjuncts to energy restriction should be a consideration to limit the amount of LBM loss. Finally, limitations to the current data are the under-representation of premenopausal populations, and to date no study has attempted to delineate differences between the responses of pre- and postmenopausal women. Considering premenopausal women are often at higher risk of weight gain after treatment, including them in future studies would be valuable for overall breast cancer management.

2.2.3 Controlled trials assessing the effect of combined exercise and dietary interventions on body composition

The combination of exercise and dietary prescription may be the most effective intervention to elicit body weight and body fat reduction while concurrently maintaining or increasing LBM. The previous sections have indicated that: exercise alone contributes to LBM gains and body fat reduction, and this occurs in the absence of body weight change; and, dietary energy restriction is related to significant reductions in body weight, however, the concurrent loss of LBM places women at greater risk of myopenia. The following review focuses on studies that have evaluated the effect of concurrent nutrition and exercise prescription. All studies that included a measure of LBM have reported maintenance (see Table 2.3), and compared to control, a reduction in body fat% and body weight have been observed (Djuric 2011, Demark-Wahnefried et al. 2008, Mefferd et al. 2007).

Of the eight published studies that combined nutrition and exercise prescription in populations with cancer, six have investigated the effects solely in breast cancer survivors (Demark-Wahnefried et al. 2008, Mefferd et al. 2007, Harris et al. 2012, Pakiz et al. 2011, Scott et al. 2013, Djuric 2011); one of which reported but was not powered to detect change in body composition (Djuric 2011); and two studies reported weight and BMI changes in survivors of mixed cancer diagnoses (Morey et al. 2009, Demark-Wahnefried et al. 2007). The mixed cancer population studies did not separate results for breast cancer populations. In addition, body composition data was limited to body weight and BMI only, thus this review will focus on the six controlled trials exclusively focused on breast cancer populations. These studies are comprised of three high quality (Demark-Wahnefried et al. 2008, Harris et al. 2012, Pakiz et al. 2011), and three neutral quality studies (Mefferd et al. 2007, Scott et al. 2013, Djuric 2011) (Table 2.3).

For those studies that included a measure of LBM, no differences were seen in LBM change within or between groups, regardless of total body weight change (Mefferd et al. 2007, Demark-Wahnefried et al. 2008, Djuric 2011). Mefferd et al (2007) reported no change in LBM in both groups measured by DEXA, while the intervention group lost total body weight (-5.7kg) and body fat (-5.5%) (Mefferd et al. 2007). Demark-Wahnefried et al (2008) reported no change in LBM for any group, however the exercise plus high fruit and veg/low fat diet group gained significantly less body fat% than the exercise alone and control groups (Demark-Wahnefried et al. 2008). Djuric et al (2011) indicated a non-significant trend for LBM increase, and body fat% decrease for the intervention group (Djuric 2011). However the study was not powered to detect body composition

changes. Furthermore, the exercise training protocols in all studies were focused on aerobic exercise and very light weight resistance training, thus LBM increases were not expected.

Differences in study design may explain these discrepancies in body fat% and body weight changes. Firstly, Mefferd et al (2007) coupled a 2000-4000kJ dietary energy restriction with home-based aerobic and supervised resistance exercise training (Mefferd et al. 2007). In contrast, Demark-Wahnefried et al (2008) did not prescribe an energy restriction and exercises were performed unsupervised. As seen in exercise-only controlled trials, supervised exercise interventions are more likely to be successful in creating body composition change. Mefferd et al (2007) included mainly postmenopausal women who finished treatment 3.5 years previous, while Demark-Wahnefried et al (2008) included only pre-menopausal women going through chemotherapy. Compared to postmenopausal women, premenopausal women who have been treated for breast cancer tend to gain more weight (Goodwin et al. 1999). In addition, chemotherapy has been shown to be associated with a reduction in sport related exercise (Irwin et al. 2005).. However, Courneya et al (2007) reported that exercise can elicit significant body composition changes during chemotherapy with supervised progressive resistance training.

Thompson et al (2010) reported clinically significant loss of LBM after dietary energy restriction alone. That Mefferd et al (2007) and Djuric et al (2011) observed concurrent body fat reduction and LBM maintenance indicates that the addition of exercise training to a dietary energy deficit is an important clinical consideration for optimal body composition outcomes.

For studies prescribing an energy deficit through diet, the magnitude of differences in body weight loss between intervention and control groups ranged from -0.85kg to -5.5kg (Mefferd et al. 2007, Pakiz et al. 2011, Scott et al. 2013, Harris et al. 2012). Differences between studies seem to be related to the amount of exercise that was prescribed. A greater magnitude of weight loss was reported in trials that prescribed >300min/week (-5.5kg) (Mefferd et al. 2007, Pakiz et al. 2011), compared to 150min/week (-3.3 to -4kg) (Harris et al. 2012), which in turn was greater than those that prescribed 120-135min/week (-1.09kg) (Scott et al. 2013). In contrast, Demark-Wahnefried et al (2008) who prescribed a high fruit and vegetable low-fat diet without energy restriction did not report any significant weight loss between groups. A lack of body weight loss for these intervention groups could be explained by a lower amount of exercise prescribed (>30min of AET, >3 times/week plus RET with therabands every other day), and adhered to (adherence: 43% and 26% for calcium plus exercise and high fruit and vegetable intake, and calcium plus exercise alone, respectively) (Demark-Wahnefried et al. 2008).

Djuric et al (2011) specifically prescribed a tailored a weight maintenance diet and therefore the <1kg change in 12 months would suggest adequate adherence to this prescription. However, the intervention group experienced a substantial within group decrease in waist girth at 6 months (-7.6cm), which was attenuated slightly at 12 months (-3.5cm) (Djuric 2011). The two other studies that reported waist girth change, compared to the control group, both reported statistically significant reductions for the intervention group (Scott et al. 2013, Harris et al. 2012, Djuric 2011). Scott et al (2013) reported a significant reduction in body weight and waist girth in the absence of body fat% change; while Djuric et al (2011) noted this but only for weight change within the intervention group. Therefore, it could be speculated that exercise preferentially targets abdominal fat, and the effect on the body is to increase muscle while fat is reduced. This effect makes physical activity important to interventions aiming to improve body composition.

A large limitation for all of these trials was the consistent lack of detail in describing the aerobic and resistance exercise programs. For the aerobic protocols, no detail of target workloads and intensity were given other than a simple outline, e.g. moderate activity for 60 minutes per day. Similarly, little description of resistance training interventions were given such that frequency, intensity and time were not made clear in the manuscript. Finally, control group protocols differed with one study providing written diet and exercise education materials (Djuric 2011), three offered no exercise and dietary energy intake advice (Mefferd et al. 2007, Pakiz et al. 2011, Scott et al. 2013, Demark-Wahnefried et al. 2008), one compared a telephone versus in-person group delivery using similar exercise and dietary recommendations (Harris et al. 2012), and one did not indicate any procedures for the control group (Scott et al. 2013).

TABLE 2.3: STUDIES EVALUATING EFFECTS OF DIET AND EXERCISE INTERVENTION ON BODY COMPOSITION

Author, Yr, Quality	Design	Population	Intervention	Body composition and adherence outcomes	Comments
Demark Wahnefried, 2008, USA Quality: + NHMRC: II Secondary outcome	3-arm RCT	N=90; Age: 41.8 (5/6) yrs; Time since Dx: Receiving CTx currently Causasion: 85%; BMI: 25.8kg/m2; Chemotherapy: 100%; Premenopausal: 100%; Taxane: 52%	6 months, phone delivered; 14 x 10-30min sessions. From 2nd cycle of chemotherapy. CaEx: Calcium rich diet + Exercise CLFEx: Calcium + Low Fat + high Fruit & Vegetable + Ex CON: Calcium attention control Exercise: Home-based: AET >30min, >3/wk (HR monitor, pace tapes); RET: Every other day – therabands Diet: Low fat: <20% energy as fat; High Fruit and veg: >5 serves/day. Calcium: 1200-1500mg/day; Measures: Baseline and 6-months. Wt, DEXA, Dietary intake (FFQ), Physical activity, QOL, HADS	No difference in weight change between groups CaEx: +2.3kg, CLFEx: +0.3, CON: +1.7 – p>0.05 Significantly less body fat% gains for CLFEx. CaEx: +1.7, CLFEx: +0.2, CON: +1.1, P<0.05 (compared to group 1 & 3, when trunk fat not included). No difference in LBM change between groups CaEx: -0.98, CLFEx: -0.29, CON: +0.69, P>0.05 Attrition: 8.8% Adherence to exercise: CLFEx: 43%, CaEx: 26%	Lean body mass not affected by intervention. Non-trunk fat mass may decrease with low fat, high fruit and vegetable + exercise. RET may not have been hard enough to elicit change, Calcium may have protein preserving effect reducing effect compared to control. Not initially powered to detect changes.
Mefferd, 2007, USA NHMRC: II Quality: O Primary outcome	RCT- wait list control	N=76; Age: 56.3 (8.2) yrs; BMI: 31.0 (4.2); Time since Dx: 3.5yrs (<1-14yrs); Caucasian: 93%; Postmenopausal: 84%; Stage: I-IIIA	16-week intervention INT: Weekly in-person meetings for 16 weeks + phone calls. Then 1/month for 6 months Diet: 500-1000kcal/day energy restriction + Exercise: AET up to 1-hr/day + a goal of 2-3/wk RET supervised (group). Self-directed goals and motivation. Measures: Baseline and 16 weeks DEXA, 7-day PAR, Lipids, No diet history	↑ weight loss for INT INT: -5.7kg (6.8); CON: -0.5kg (0.6), P<0.05 ↑ loss of body fat% for INT INT: -5.5 (-15), CON: -1.4 (-3.5), P<0.01 No change in LBM for either group INT: +0.1kg, CON: 0. Leg and trunk fat decreased in INT group >80% attendance to sessions	Significant fat mass loss with no loss of LBM. Population may not be experiencing myopenia that long after treatment. No data on adherence/ progression of exercise/ type of resistance training
Pakiz Italy, 2012 NHMRC II Quality: +ve Primary outcome	2-arm RCT 2:1 INT:Con	N=85 INT: n=56 vs CON: n=29 Stage I-IIIa Dx: within last 14 yrs; Age: 33-71yrs Caucasian:94%,	12 month intervention INT: Exercise: Goal 1hr/day moderate intensity Diet: 500-1000kcal/day energy deficit Contact: In-person group - weekly for 4 mths, 1/month follow-ups from 5-12 months. Individual phone calls weekly for 1 month, fortnightly (month 2-3), monthly from 4-12 month CON: Wait-list control group Measures: 0, 16 & 52 weeks Body comp: DEXA 7-day physical activity recall instrument Step test – 1st 15s recovery	↑ weight loss for INT INT: -5.7kg (3.5), CON: -0.2kg (4.1), p<0.0001 ↑ change in body fat% for INT INT: -4.5 (3.8), CON: -0.9 (2.3), p<0.0001 ↑ change in waist for INT INT: -7.1cm (6.4), CON: -2.5cm (7.7) Improved HR/30s for INT: Step test Increased PA for INT group (hrs/day exercise) PA: +2.2hrs vs +0.3hrs	Intervention improved weight, body fat% and waist girth, physical activity and fitness. No report of LBM Decreases in TNF-a for both groups, ?not related to fat tissue Discussion looked to single out INT group results, while disregarding results of control group

Djuric (Djuric 2011) 2 arm RCT NHMRC: II Quality: Neutral Not powered for body comp	2-arm RCT	N=40, >18yrs, Stage I-IIIA, BMI: 26.1-28.1, Scheduled for Chemo or within 2 weeks of starting. Stable Wt (within 2.5kg for last 2 months)	12 month intervention INT: Written information and MI telephone counselling. Diet: High fruit and veg/low fat diet with weight control. Fat and Fast Food Counters + semi-tailored meal plan for weight stability. Exercise: AET: Goal - 30min mod-vig/day CON: Written information as above. No telephone MI sessions Contact: weekly for 2 weeks, bi weekly for 5 months, monthly for the last 6 months 25% drop out – higher in INT Measures: 0, 6 12mths Body comp: Wt, Ht, DEXA, Waist and hip PAQ from WHI, QOL: FACT-B Dietary: Dietary screeners (fruit and veg and energy from fat screener) + one-off 24 hour unannounced recall. Compared to a modified 5-pass recall. HDL, TG, CRP, C-peptide, IGF-1, IGFBP-3 Total carotenoids	No significant change in body weight at 6 or 12 months, compared to baseline INT: 71.7kg, +0.5kg, -0.8kg, CON: 71.3kg, -0.8kg, +0.7kg - NS No significant change in LBM after 12 months INT: +1.4%, CON: -1.2% No significant changes in body fat% at 12 mths INT: -1.7%, CON: +1.2% - NS Significant within group decrease in waist for INT at 6 months 6 month change: INT: -7.6cm ($p<0.05$), CON: -0.6cm 12 month change from baseline: INT: -3.5cm, CON: +2.7cm No change in CRP, Blood pressure.	Completers – higher F&V and higher carotenoids than non-completers. CRP higher at baseline in CON Effect of had started/not started chemo: - no diff in anthro or bloods - higher physical scores and breast cancer specific subset for non-starters. Fruit and veg screener predicted more fruit and veg than the recall Increase in physical activity, QOL and
Harris 2012 NHMRC: IIIi Quality: +ve Primary – Weight change	2-arm quasi-experimental	N=35; 52.8kg, Wt:86.1, 71.4% postmenopausal, 80% Caucasian, No diff at baseline	Phase I: 6 months; Phase 2: 6-12 months In-Person (IP) INT: 16 sessions (60-90min). PA: 150min/wk mod exercise, 500-1000Cal energy deficit (aim 0.5 to 1kg/wk Wt loss) Phase 2: Monthly contact by trained interventionist (promote further loss or maintenance) Phase 1: Tele-INT (Tele): 1/wk contact (15-60min sessions) (TrestleTree, Inc), similar but tailored intervention with similar guidelines to above Phase 2: Monthly contact by TrestleTree Measures: 0, 6, 12 months Body composition: Wt, BMI, Waist Bloods: LDL, HDL, TChol, TGs, BGLs Blinded: assessment and intervention teams	Significant weight loss for both groups after 6 months. No diff between groups IP: -3.3kg, p=0.002 Tele: -4kg, p=0.01 Significant weight regain at 12 months for IP, with weight maintenance for Tele group. In-person: +1.3kg, p=0.009 Tele: -1.0kg, p=0.185 Between groups: p=0.056 Significant decrease in waist for both groups after 6 months. No diff b/w groups. In-Person: -3.4cm, $p<0.05$ Tele: -5.6: -5.6cm, $p<0.05$ Decrease in TG IP Decrease in LDL for Tele compared to IP.	Mode of delivery did not have a bearing on results. Individual consultation may be inherently advantageous Each 1kg of weight lost is a 16% reduction in Diabetes risk

Scott 2013 NHMRC: II Quality: Neutral Primary outcome – Body weight	2-arm RCT	N=90 (47 &43); 3-18 months post Rx; Age: 55.6-55.9yrs; Wt: 78-83.2kg; Caucasian: 98%; CTx: 54-57%; RTx: 85-81%; Tamoxifen: 49-51%; AI: 30-26%; Postmeno: 67.7%;	24 weeks INT - Sessions 3/wk supervised exercise and diet education sessions. Exercise: 30min AET @ 65-85% + RET: 10-15min resistance bands, hand weights, stability balls. Individualised diet counselling: 2500kJ deficit + weekly group sessions CON: Unknown Measures: 0 and 24wk - ITT Body composition: BIA – body fat%, Wt, waist, hip Fitness: Estimated Aerobic fitness, BP QOL: FACT-B Diet: 3-day diet diaries Bloods: Testosterone, SHBG, BGLs, hs-CRP, TChol, HDL, Estrone, Estradiol, insulin, IGF-1, IGFBP-1 & 3, Leptin	80% adherence ITT: Borderline reduction in weight for INT vs CON at 24 wks. INT: -1.09kg (IQR: -0.15 to -2.9kg) CON: -0.4kg (IQR: 0.7 to -1.8kg) P=0.07, between groups When outliers (>3 SD from mean) were removed: INT: -1.25 (IQR: -0.26 to -2.93kg) CON: -0.4 (IQR: 0.73 to -1.72kg) P=0.03 No change in BF% was seen Significant decrease in waist for INT compared to CON Adj mean difference: -3.32 (95% CI -1.53 to -5.11), p=0.001 Fitness: Improved aerobic fitness, diastolic blood pressure QOL: Improved FACT-B: +6 (p=0.004), Subscale: +2 (p=0.007) between groups	No description of control activity Low quality body composition measure No LBM measure `

INT: Intervention group; CON: Control group; ITT: Intention to treat; RCT: Randomised controlled trial; Ca: Cancer; Sx: Surgery, CTx: Chemotherapy; RTx: Radiotherapy; AIs: Aromatase Inhibitors; Rx: Treatment; HR: Heart rate; AET: Aerobic exercise training; RET: Resistance exercise training; Wt: Weight; Ht: Height; LBM: Lean body mass; DEXA: Dual energy X-ray absorptiometry; QOL: Quality of life; FFQ: Food frequency questionnaire; PA: Physical activity; CV: Cardiovascular; Time since Dx: time since diagnosis; IGF: Insulin like growth factor; HOMA: Homeostasis Model Assessment; INT: Intervention group; CON: Control group; ITT: Intention to treat; mths: Months; 7-Day PAR: 7-day Physical Activity Record; FACT-B: Functional Assessment during Cancer Therapy – Breast; CRP: C-Reactive Protein; HADS: Hospital Anxiety and Depression Score. PAQ: Physical Activity Questionnaire; WHI: Women's Health Initiative(Irwin et al. 2011)

In summary, the lack of high quality studies evaluating the combined effects of exercise and dietary interventions on body composition in breast cancer survivors currently precludes the ability to derive firm conclusions regarding optimal interventions manipulating body composition. However, initial findings indicate that dietary energy restriction coupled with combined aerobic and resistance exercise may evoke a clinically significant reduction in body weight, body fat%, or waist girth with concurrent LBM maintenance. Similarly, diet prescriptions aiming to maintain weight in conjunction with exercise may reduce body fat gains during chemotherapy. Future studies should include participants of similar time post diagnosis, with a specific focus on those who are completing, or have completed chemotherapy in the last 12 months, as this may be both the most teachable moment for lifestyle change (Harvie 2010), and also the most critical time to prevent change in weight (Makari-Judson et al 2007) Finally, it is important to note that while attempts to create weight loss and LBM maintenance have been successful in these studies, the long term effects of this body composition change are currently unknown in this population. By observing other populations, reductions in body fat/waist, and LBM retention are likely to promote greater metabolic health. However at this point, the effect of relatively fast and substantial weight loss on breast cancer and overall mortality and morbidity has not been studied. Some evidence does indicate a benefit for moderate weight loss through diet and exercise changes in a population of women with breast cancer (Ligibel and Goodwin 2012). Thus a smaller reduction in body weight comprised mainly of body fat is the best clinical target given the current evidence. A combination of healthy eating without energy restriction plus exercise prescription is most likely to create this type of change.

2.3 Published manuscript #1

The following published manuscript was written as a brief review of the information covered above. The focus of the article was the influence of LBM on breast cancer, and in turn, the influence of breast cancer on LBM. Up until this point, the majority of breast cancer research has focused on fat tissue. However, evidence for muscle-fat cross talk relating to the immune and hormone regulation make LBM a critical consideration when discussing metabolic function and disease outcomes (Pedersen and Febbraio 2012, Brandt and Pedersen 2010). The manuscript gives a short overview of the effects of exercise and diet on LBM change, and then considers how these effects could be optimised through specific nutrients. Here the possible benefits of LCn-3s are introduced. These benefits are then discussed in detail in the following section of this chapter.

BODY COMPOSITION AND BREAST CANCER – THE ROLE OF LEAN BODY MASS

Cameron McDonald, Judith D Bauer and Sandra Capra
University of Queensland, Queensland.
Email: cameron.mcdonald1@uqconnect.edu.au

Abstract

Breast cancer risk and outcomes for breast cancer survivors are known to be influenced by body composition. A wealth of literature surrounds the function and role of fat tissue, however considerably less is known regarding lean body mass and its functional role in immune, hormonal and metabolic regulation in breast cancer aetiology. This review outlines findings relevant to lean body mass before, and following breast cancer diagnosis. A paucity of research exists regarding lean body mass and breast cancer risk. However, post-diagnosis lean body mass losses are commonly reported and a concern for ongoing co-morbidity after treatment. A comprehensive mechanism for sarcopenic obesity in breast cancer survivors is currently unknown. However, findings from other disease states indicate that the effects of chronic inflammation and/or an increase in sedentary activity may partly explain the exaggerated losses of lean body mass. Exercise has been a successful intervention for attenuating lean body mass losses after treatment, while weight loss through energy restriction may exacerbate breast cancer related sarcopenia. Combining exercise with dietary intervention to optimise lean body mass may be ideal; however there is insufficient evidence for this at present. Similarly, the role of functional food supplements, such as omega-3 fatty acids and essential amino acids, may aid lean body mass maintenance through anti-inflammatory action and increased muscle protein synthesis.

There were 1.15 million new cases of breast cancer diagnosed worldwide in 2002,[1] while in Australia alone, 12,600 new cases are diagnosed each year and at the end of 2006 there were 144,000 breast cancer survivors country-wide.[2] Significant advances in research have increased our understanding of predisposing factors and improved the management of breast cancer, resulting in a five-year survival rate of 88% and a one-year survival of 97%.[2]

Over the last three decades, numerous studies and meta-analyses have established a relationship between body composition and breast cancer aetiology and prognosis.[3-6] Postmenopausal breast cancer risk has a positive correlation with body mass index (BMI),[3] while a lower BMI[3] but high waist to hip ratio (WHR) is associated with an increased risk of premenopausal breast cancer.[4,5] At the time of diagnosis, a higher BMI and WHR are both related to poorer prognosis, irrespective of menopausal status.[6]

Due to the strong correlation found between BMI, WHR and body fat mass, investigations have focused on the function of fat tissue in breast cancer aetiology with specific reference to its influence over sex hormone balance, endocrine function, insulin and insulin-like growth factors and adipokine expression.[7] More recently, better understanding of the function of lean body mass (LBM) indicates that it too exerts a powerful endocrine, immune and hormonal influence within the body.[8]

For breast cancer survivors, simultaneous LBM loss with fat tissue accumulation, known as sarcopenic obesity, is common.[9-11] The complete aetiology of LBM loss in this population is unclear, however it appears to be associated with poorer metabolic outcomes, such as earlier onset of cardiovascular disease and metabolic syndrome related diseases.[8,12,13] In addition, LBM has been shown to be a positive predictor of survival in chronic heart failure,[14] chronic kidney disease,[15] chronic obstructive pulmonary disease,[16] and cancer cachexia.[17] Evidence from these populations suggest that LBM loss may in part be related to inflammatory mediators present as a result of the disease state and treatment.[17,18]

The purpose of this review is: to provide a brief outline of findings related to LBM before and after breast cancer diagnosis; to explore the role of inflammation in LBM loss in breast cancer survivor populations; and review the established and potential roles of exercise and dietary intake in LBM maintenance specific to the breast cancer survivor population.

Search criteria

A literature search was carried out using MEDLINE and Pubmed databases. Selected studies and review articles were hand-searched for additional relevant references. Key terms used included: breast cancer (breast neoplasms, cancer of the breast, breast cancer survivor, breast neoplasm risk); body composition (percentage body fat, muscle mass, lean body mass, skeletal muscle, body composition); exercise (physical activity, resistance training, aerobic training); diet (energy intake, omega-3 fatty acids, diet therapy, caloric/energy restriction). Additional search criteria included, subjects >18 years of age, non-metastatic breast cancer survivors and articles published in English. Included articles were those that reported body fat composition and/or lean body mass in relation to: breast cancer risk (all study designs included); time after breast cancer diagnosis (all prospective and retrospective cohort studies, case series, non-randomised and randomised studies); and diet and exercise, or combined interventions post breast cancer diagnosis (all non-randomised and randomised control trials).

LBM prior to breast cancer diagnosis

There is a lack of studies prospectively assessing LBM in association with breast cancer risk using sensitive measures such as dual-energy X-ray absorptiometry, CT scanning, densitometry or bioelectrical impedance. Of the studies that could be located, two prospective cohorts consisting entirely of postmenopausal women, have reported mixed results for the effect of LBM on breast cancer risk as assessed by bioelectrical impedance.[19,20] In a Dutch postmenopausal population with a median of six years follow-up, each 1kg/m^2 increase in LBM-to-height ratio (LBM divided by height squared) was positively associated with breast cancer risk, with seemingly no effect from body fat to height ratio.[20] This differed somewhat to a postmenopausal Australian cohort measured at baseline and again after nine years.[19] Each 10kg increase in absolute lean body and fat mass, and 10cm increase in waist circumference, were associated with increased breast cancer risk. However, when results were stratified for time since onset of menopause and history of hormone replacement therapy (HRT), a significant effect was only found for those who had experienced menopause more than 15 years before assessment, and in never-users of HRT.[19-21]

These results are not surprising, as it is well established that adult weight increases and higher BMI values are significantly associated with postmenopausal breast cancer risk.[3,21] Considering normal weight gain in healthy adult populations involves a simultaneous increase in LBM and fat mass,[22] the association between breast cancer risk and absolute LBM in these studies may be secondary to the effects of significant long-term total body weight and fat mass gain during adulthood.

In contrast to the above findings, when the ratio of fat to skeletal muscle mass was measured at or shortly following diagnosis in a Uruguayan case-control study, a higher value for fat-to-muscle ratio was more indicative of a breast cancer diagnosis.[23] Compared to the lowest (1st) quartile of fat-to-muscle ratio, both 3rd and 4th quartiles had an odds risk of 4.86 and 6.09 ($p<0.0001$) independent of BMI and menopausal status. The authors noted that to maintain skeletal muscle mass at a level that was protective, regular exercise was mandatory. Alternatively, these results may indicate the importance of active lean tissue and its influence over immune and hormonal regulation.[24] Caution in interpretation of these data is required. Limitations regarding the body composition measurement methodology used, and the applicability of findings to populations in developed countries are not clear.

To date, few meaningful relationships between LBM and risk of breast cancer have been uncovered. Current evidence suggests that the effect of LBM may be secondary to total weight and fat mass gains prior to diagnosis. More prospective studies using accurate and repeated measures of body composition, along with markers of muscle function, are required to further elucidate the protective or predisposing effect of LBM and breast cancer risk.

Pattern of LBM changes after breast cancer treatment

Sarcopenic weight gains are common after treatment for breast cancer.[10] Over the five years following active treatment, 50-100% of survivors have been shown to increase total weight,[10,11] with the probability of re-attaining their pre-diagnosis weight being inversely associated with initial post-treatment weight gains.[12] LBM growth accounts for 20-40% of total weight gains in disease free populations.[22] Studies of breast cancer survivors have shown that more than one year after chemotherapy, total fat mass gains of 2.4kg to 6.7kg were accompanied by LBM losses of -0.4kg to -1.7kg, respectively.[9,25] Women who seemingly maintain their weight in the years after treatment still undergo these adverse changes, such that LBM losses match increases in adipose tissue.[26] Factors that are linked with more exaggerated changes include premenopausal status at diagnosis, experiencing treatment related menopause,[27] receiving chemotherapy compared to no chemotherapy, a lower BMI at diagnosis and those who are least physically active after treatment.[28] The sarcopenic pattern is still prevalent, albeit of smaller magnitude in postmenopausal breast cancer populations.[25, 29]

In regards to timing of LBM changes, the most significant changes are seen during adjuvant chemotherapy and in the 6 to 12 months following this.[9,25,29,30] By observing control groups in large randomised trials, the rate of sarcopenic weight gain seems to normalise two to four years post diagnosis,[31-34] however total weight increases can still occur after this point.[12]

LBM losses with concurrent fat and total weight gains are associated with metabolic dysfunction including impaired glucose metabolism,[13] high triglyceride levels,[35] and chronic inflammation in healthy and diseased populations.[8] While the function of fat tissue has been a focus of previous interventions aimed at breast cancer survivors, LBM should be evaluated more closely in future, as it is known to be a large contributor to glucose disposal,[8] triglyceride oxidation and, when stimulated through exercise, can exert systemic anti-inflammatory effects.[36]

Contributors to LBM losses

Studies assessing moderators of weight change during treatment (local surgery and radiotherapy, with or without chemotherapy) have not conclusively explained the reasons for the higher than expected total weight gains and the sarcopenic nature of the body composition changes.[9,25,27,37,38] The role of both resting metabolic rate and energy intake do not fully explain the magnitude of weight change after treatment.[9,27] It is thought that any increases in fat mass are sufficient to mask the resting metabolic rate reduction associated with LBM losses,[9] while weight gains have been observed even after a reduction in energy intake.[27] In contrast, lower levels of physical activity have been associated with increased weight,[38] however total weight gains still seem to be greater than predicted after accounting for the reduction in energy expenditure associated with decreased physical activity.[25] Therefore, auxiliary mechanisms other than those relating to

conventional energy balance, such as chronic inflammation metabolic disturbances related to sedentary activity, may partly explain the exaggerated changes in LBM.

Systemic inflammation has proven to be a strong inhibitor of muscle protein synthesis and increased muscle protein degradation in ovarian, gastroesophageal and pancreatic cancers.[39,40] A full review of these mechanisms can be found elsewhere.[40] In brief, increased circulating levels of inflammatory cytokines such as tumour necrosis factor (TNF-)alpha and interleukin-6 (IL-6), and increased genetic expression of inflammatory markers through nuclear factor-kappa B (Nf-ƙB), stimulate muscle degradation while inhibiting muscle protein synthesis.[40] At least one prospective study revealed that elevated levels of inflammatory markers have been positively associated with body mass accumulation in healthy populations.[41]

Direct associations between LBM changes and inflammatory markers have not yet been made in breast cancer survivor populations. Elevated levels of acute phase inflammatory markers, C-reactive protein and serum amyloid A, have been correlated with increased fatigue,[42] increased incidence of cardiovascular disease, insulin resistance,[43] and mortality independent of BMI, stage of disease and race.[44] Cytokines generated from active LBM (particularly skeletal muscle), known as myokines,[45] contribute to the anti/inflammatory balance of the body.[8] While the muscle-fat cytokine interplay has not been fully elucidated, numerous studies have confirmed that muscle activity has a significant anti-inflammatory influence on the systemic cytokine milieu, and further research may develop mechanisms that increase the importance of functional LBM in healthy and breast cancer populations.[24]

A reduction in physical activity and an increase in sedentary activity are common after breast cancer diagnosis.[28] Increased sedentary time, such as sitting or lying down, has been related to increased adiposity in breast cancer populations.[46] This phenomenon can be explained through an increase in abdominal fat deposition, decreased insulin sensitivity,[35] decreased triglyceride oxidation,[35] and an inhibition of muscle synthesis,[47] following muscle deactivation related to physical inactivity. Decreased energy expenditure plus the metabolic disturbances associated with physical inactivity, may partially explain discrepancies in predicted and actual weight gains found in breast cancer survivors.

Inflammation and sedentary activity related changes in metabolism have a significant role in LBM physiology. More research is needed to fully elucidate exact physiological mechanisms even in healthy populations, however compelling evidence indicates that regularly stimulated as opposed to dormant LBM may be closely related to LBM changes.[13, 48]

Influences of exercise and diet on LBM

Diet and physical activity interventions have had a significant impact on body composition changes in breast cancer survivors despite their disappointing influence on LBM following treatment.

Regular exercise in the well population has been shown to reduce breast cancer risk by 25-30%,[49] and after diagnosis, total mortality by ~40%, breast cancer mortality by 34%, and breast cancer recurrence by 24%.[50] Therefore, increased physical activity is recommended for healthy populations and breast cancer survivors alike.

With respect to LBM, randomised control trials that involved resistance training have shown 0.5 to 0.88kg LBM increases over 8 to 26 weeks.[51-53] In a population that typically loses muscle mass, aerobic exercise during and after treatment when compared to no intervention, has been shown to attenuate and sometimes reverse LBM losses.[32, 33] However, a recent meta-analysis of randomised control trials notes only body fat percentage is consistently improved by aerobic exercise in this population.[54] As well as absolute LBM growth, improvement of muscle function in conjunction with smaller absolute LBM growth is an important outcome in this population. A landmark randomised control trial by Schmitz et al (2009) investigated the effect of year long, twice weekly resistance training on outcomes relating to lymphoedema. The study did not detect a significant change in LBM compared to control. However upper and lower body strength increased by 29% and 32% respectively in the intervention group, compared to 4% and 8% respectively in the control.[31] Similarly, VO_2 max was disproportionately improved after aerobic exercise training compared to the relatively small improvements of body composition.[54,55] Considering the varying abilities of individuals of different body shapes and genetic predisposition to increasing absolute LBM, functional outcomes may give a more consistent insight into physiological improvement of LBM. Muscle strength has been shown to be a better predictor of mortality than muscle mass in ageing populations,[56] VO_2 max has long been an independent marker of mortality regardless of body composition in other populations,[57] and evidence shows that exercise training and muscle contraction exerts anti-inflammatory effects through myokine production.[24] While the data regarding outcomes and muscle function is lacking in breast cancer survivors, these consistent relationships in otherwise not dissimilar populations are suggestive of similar links in breast cancer populations.

Dietary interventions for breast cancer survivors have shown successful weight loss through energy restriction,[58-61] and with mixed results after low fat and high fruit and vegetable consumption.[62,63] Randomised control trials assessing weight loss through energy restriction in breast cancer survivors have resulted in 3.3 to 9.5kg weight loss over 6 to 12 months.[59-61] However, there has been little focus on lean mass maintenance in these studies. In otherwise healthy overweight and obese populations, weight loss through energy restriction without exercise inevitably results in losses of both fat and LBM.[60,64,65] A recent randomised control trial evaluated the efficacy of low carbohydrate or low fat diets for weight loss in breast cancer survivors and their potential hazard to LBM.[60] Similar weight loss was found for each group, however, while body fat percentage, metabolic markers and C-reactive protein decreased, a classification of sarcopenia categorised

by appendicular LBM (<5.67kg/m^2), measured by dual-energy X-ray absorptiometry, increased from 8% to 18% within the study cohort.[60] Considering the known link between breast cancer survival and the loss of LBM after treatment, this study is the first in this population that clearly indicates the need for additional interventions to attenuate LBM during weight loss.

Combining exercise and dietary restriction for breast cancer survivors has shown promise in attenuating LBM loss during total body weight loss.[66] Some studies have been underpowered or have failed to measure LBM,[67-69] leaving the need for more research into a model that has been useful in non-breast cancer populations.[65] Apart from exercise, anti-inflammatory nutrients may have utility in this population when addressing LBM maintenance. Long chain omega-3 fatty acids (LCn-3 FAs) through anti-inflammatory and mitochondrial influence, are associated with protein sparing and increased fat oxidation in overweight populations,[70-72] and LBM attenuation in cancer cachexia.[39,73] In conjunction with exercise, LCn-3FAs supplementation has shown to exert more powerful effects again on fat oxidation and LBM growth.[71] Substantial literature supports the ability of LCn-3FAs to reduce inflammation through many of the pathways associated with LBM loss.[74-76] An Australian study is currently underway investigating these relationships within a breast cancer survivor cohort. Another potential group of nutrients that show promise in LBM preservation are supplemented essential amino acids. Emerging findings indicate that essential amino acids, when dosed appropriately, may independently stimulate muscle protein synthesis.[77] Supplementation has improved LBM in both chronic heart failure and older female populations,[78,79] and has a theoretical potential in breast cancer populations.

Conclusions

Adipose tissue has long been a focus of breast cancer aetiology and management. While little published research exists, recent insights regarding the role of LBM in inflammatory, immune and hormonal balance indicate an intriguing avenue for improving breast cancer outcomes. Sarcopenic weight gains during and after breast cancer treatment are not fully understood, however inflammatory regulation, inactivation of muscle tissue through sedentary activity and muscle-fat communication via endocrine pathways may provide further explanation of these adverse changes. Regardless of the incomplete physiological understanding, exercise interventions during and after treatment are effective in attenuating and reversing LBM losses in breast cancer survivors. Perhaps more importantly, it has been shown to dramatically improve muscle function in breast cancer populations. In contrast, dietary energy restriction alone is effective in reducing weight, however, the concurrent loss of LBM during weight loss may expose survivors to more severe sarcopenic changes. Optimal management of body composition is still under investigation, however conclusions from other populations would indicate a combined diet and exercise approach is best. Finally, a potential role exists for specific dietary supplements that address chronic inflammation and inhibition of muscle protein synthesis likely present in breast cancer survivors.

References

1. Parkin DM, Bray F, Ferlay J, Pisani P. Global Cancer Statistics, 2002. CA Cancer J Clin. March 1 2005;55(2):74-108.
2. Australian Institute of Health and Welfare & National Breast and Ovarian Cancer Centre. Breast cancer in Australia: an overview, 2009. Cancer series no. 50. 2009;Cat. no. CAN 46. Canberra: AIHW.
3. Renehan AG, Tyson M, Egger M, Heller RF, Zwahlen M. Body-mass index and incidence of cancer: a systematic review and meta-analysis of prospective observational studies. Lancet. 2008;371:569-578.
4. Connolly BS, Barnett C, Vogt KN, Li T, Stone J, Boyd NF. A Meta-Analysis of Published Literature on Waist-to-Hip Ratio and Risk of Breast Cancer. Nutrition and Cancer. 2002;44(2):127-138.
5. Harvie M, Hooper L, Howell AH. Central obesity and breast cancer risk: a systematic review. Obes Rev. 2003;4(3):157-173.
6. Protani M, Coory M, Martin JH. Effect of obesity on survival of women with breast cancer: systematic review and meta-analysis. Breast Cancer Res Treat. 2010;123:627-635.
7. Roberts DL, Dive C, Renehan AG. Biological Mechanisms Linking Obesity and Cancer Risk: New Perspectives. Annual Reviews. 2010;61:301-316.
8. Mourtzakis M, Bedbrook M. Muscle atrophy in cancer: a role for nutrition and exercise. Applied Physiology, Nutr Metab. 2009;34(5):950-956.
9. Demark-Wahnefried W, Peterson BL, Winer EP, Marks, L, Aziz N, Marcom P, et al. Changes in Weight, Body Composition, and Factors Influencing Energy Balance Among Premenopausal Breast Cancer Patients Receiving Adjuvant Chemotherapy. J Clin Oncol. 2001;19:2381-2389.
10. Rooney M, Wald A. Interventions for the Management of Weight and Body Composition Changes in Women With Breast Cancer. Clin J Oncol Nurs. 2007;11(1):41-52.
11. Harvie M. The Importance of Controlling Body Weight After a Diagnosis of Breast Cancer: The Role of Diet and Exercise in Breast Cancer Patient Management. 2010:73-96.
12. Nichols HB, Trentham-Dietz A, Egan KM, et al. Body Mass Index Before and After Breast Cancer Diagnosis: Associations with All-Cause, Breast Cancer, and Cardiovascular Disease Mortality. Cancer Epidemiology. Biomarkers & Prevention. 2009;18:1403-1409.
13. Healy LA, Ryan AM, Carroll P, Ennis D, Crowley V, Boyle T, et al. Metabolic Syndrome, Central Obesity and Insulin Resistance are Associated with Adverse Pathological Features in Postmenopausal Breast. Clin Oncol. 2010;22(4):281-288.
14. Levine B, Kalman J, Mayer L, Fillit HM, Packer M. Elevated Circulating Levels of Tumor Necrosis Factor in Severe Chronic Heart Failure. N Engl J Med. 1990;323(4):236-241.
15. Horwich TB, Fonarow GC. Cardiovascular and survival paradoxes in dialysis patients: Reverse Epidemiology beyond Dialysis Patients: Chronic Heart Failure, Geriatrics, Rheumatoid Arthritis, COPD, and AIDS. Seminars in Dialysis. 2007;20(6):549-553.
16. Sin DD, Man SF. Systemic inflammation and mortality in chronic obstructive pulmonary disease. Can J Physiol Pharmacol. 2007;85(1):141-147.
17. Argiles JM, Busquets S, Felipe A, Lopez-Soriano FJ. Abstract. Molecular mechanisms involved in muscle wasting in cancer and ageing: cachexia versus sarcopenia. The International Journal of Biotechnology & Cell Biology. 2005;37:1084-1104.
18. Schaap LA, Pluijm SMF, Deeg DJH, Visser M. Inflammatory Markers and Loss of Muscle Mass (Sarcopenia) and Strength. Am J Med. 2006;119(6):526. e529-526.e517.
19. MacInnis RJ, English DR, Gertig DM, Hopper JL, Giles GG. Body Size and Composition and Risk of Postmenopausal Breast Cancer. J Cancer Epidemiol Prev. 2004;13(12):2117-2125.
20. Mellemkjaer L, Bigaard J, Tjonneland A, et al. Body Composition and Breast Cancer in Postmenopausal Women: A Danish Prospective Cohort Study[ast]. Obesity. 2006;14(10):1854-1862.
21. Lahmann PH, Lissner L, Gullberg B, Olsson H, Berglund G. A prospective study of adiposity and postmenopausal breast cancer risk: The Malmö diet and cancer study. Int J Cancer. 2003;103(2):246-252.
22. Forbes GB, Brown MR, Welle SL, Lipinski BA. Deliberate overfeeding in women and men: energy cost and composition of the weight gain. Br J Nutr. 1996;56:1-9.
23. Ronco AL, Boeing H, De Stefani E, Schulz M, Schulze M, Pischon T. A case-control study on fat-to-muscle ration and risk of breast cancer. Nutrition and Cancer. 2009;61(4):466-474.
24. Pedersen BK. The diseasome of physical inactivity – and the role of myokines in muscle–fat cross talk. J Physiol. 2009;587(23):5559-5568.
25. Harvie MN, Campbell IT, Baildam A, Howell A. Energy Balance in Early Breast Cancer Patients Receiving Adjuvant Chemotherapy. Breast Cancer Res Treat. 2004;83(3):201-210.
26. Kutynec CI, McCargar L, Barr SI, Hislop TG. Energy balance in women with breast cancer during adjuvant treatment. J Am Diet Assoc. 1999;99:1222-1227.
27. Goodwin PJ, Ennis M, Pritchard KI, et al. Adjuvant Treatment and Onset of Menopause Predict Weight Gain After Breast Cancer Diagnosis. J Clin Oncol. 1999;17:120-129.
28. Irwin ML, Crumley D, McTiernan A, et al. Physical activity levels before and after a diagnosis of breast carcinoma. Cancer. 2003;97(7):1746-1757.

29. Aslani A, Smith RC, Allen BJ, Pavlakis N, Levi JA. Changes in body composition during breast cancer chemotherapy with the CMF regimen. Breast Cancer Res Treat. 1999;57(3):285-290.
30. 30. Battaglini C, Bottaro M, Dennehy C, et al. The effects of an individualized exercise intervention on body composition in breast cancer patients undergoing treatment. Sao Paulo Medical Journal. 2007;125(1):22-28.
31. Schmitz KH, Ahmed RL, Troxel A, et al. Weight Lifting in Women with Breast-Cancer-Related Lymphedema. N Engl J Med. 2009;361(7):664.
32. Irwin ML, Alvarez-Reeves M, Cadmus L, et al. Exercise Improves Body Fat, Lean Mass, and Bone Mass in Breast Cancer Survivors. Obesity. 2009;17(8):1534-1541.
33. Matthews C, Wilcox S, Hanby C, et al. Evaluation of a 12-week home-based walking intervention for breast cancer survivors. Supp Care Cancer. 2007;15(2):203-211.
34. Thomson CA, Rock CL, Giuliano AR, et al. Longitudinal changes in body weight and body composition among women previously treated for breast cancer consuming a high-vegetable, fruit and fiber, low-fat diet. Eur J Nutr. 2005;44(1):18-25.
35. Hamilton MT, Hamilton DG, Zderic TW. Role of Low Energy Expenditure and Sitting in Obesity, Metabolic Syndrome, Type 2 Diabetes, and Cardiovascular Disease. Diabetes. November 2007 2007;56(11):2655-2667.
36. Flynn MG, McFarlin BK, Markofski MM. State of the Art Reviews: The Anti-Inflammatory Actions of Exercise Training. American Journal of Lifestyle Medicine. May/June 2007 2007;1(3):220-235.
37. Kumar N, Allen KA, Riccardi D, et al. Fatigue, weight gain, lethargy and amenorrhea in breast cancer patients on chemotherapy: is subclinical hypothyroidism the culprit? Breast Cancer Res Treat. 2004;83(2):149.
38. Demark-Wahnefried W, Hars V, Conaway MR, et al. Reduced rates of metabolism and decreased physical activity in breast cancer patients receiving adjuvant chemotherapy. Am J Clin Nutr. 1997;65(5):1495-1501.
39. Fearon KCH, von Meyenfeldt MF, Moses AGW, et al. Effect of a protein and energy dense n-3 fatty acid enriched oral supplement on loss of weight and lean tissue in cancer cachexia: a randomised double blind trial. Gut. October 2003 2003;52(10):1479-1486.
40. Al-Majid S, Waters H. The Biological Mechanisms of Cancer-Related Skeletal Muscle Wasting: The Role of Progressive Resistance Exercise. Biol Res Nurs. 2008;10(1):7-20.
41. Duncan BB, Schmid MI, Chambless LE, Folsom AR, Carpenter M, Heiss G. Fibrinogen, Other Putative Markers of Inflammation, and Weight Gain in Middle-aged Adults—The ARIC Study. Obes Res. 2000;8(4):279-286.
42. Collado-Hidalgo A, Bower JE, Ganz PA, Cole SW, Irwin MR. Inflammatory Biomarkers for Persistent Fatigue in Breast Cancer Survivors. Clin Cancer Res. May 1, 2006 2006;12(9):2759-2766.
43. Rose DP, Komninou D, Stephenson GD. Obesity, adipocytokines, and insulin resistance in breast cancer. Obes Rev. 2004;5(3):153-165.
44. Pierce BL, Ballard-Barbash R, Bernstein L, et al. Elevated Biomarkers of Inflammation Are Associated With Reduced Survival Among Breast Cancer Patients. J Clin Oncol. July 20, 2009 2009;27(21):3437-3444.
45. Pedersen BK. State of the Art reviews: Health benefits related to exercise in patients with chronic low systemic inflammation. American Journal of Lifestyle Medicine. 2007;1:289-298.
46. Lynch BM, Dunstan DW, Healy GN, Winkler E, Eakin E, Neville O. Objectively measured physical activity and sedentary time of breast cancer survivors, and associations with adiposity: findings from NHANES (2003–2006). Cancer Causes Control. 2010;21:283-288.
47. Biolo G, Ciocchi B, Stulle M, et al. Metabolic consequences of physical inactivity. J Ren Nutr. 2005;15(1):49-53.
48. Emaus A, Veierød M, Tretli S, et al. Metabolic profile, physical activity, and mortality in breast cancer patients. Breast Cancer Res Treat. 2010;121(3):651-660.
49. Friedenreich CM, Cust AE. Physical Activity and breast cancer risk: impact of timing, type and dose of activity and population subgroup effects. Br J Sports Med. 2008;42:636-647.
50. Ibrahim E, Al-Homaidh A. Physical activity and survival after breast cancer diagnosis: meta-analysis of published studies. Med Oncol. 2010:1-13.
51. Courneya KS, Segal RJ, Mackey JR, et al. Effects of Aerobic and Resistance Exercise in Breast Cancer Patients Receiving Adjuvant Chemotherapy: A Multicenter Randomized Controlled Trial. J Clin Oncol. October 1, 2007 2007;25(28):4396-4404.
52. Herrero F, San Juan AF, Fleck SJ, et al. Combined Aerobic and Resistance Training in Breast Cancer Survivors: A Randomized, Controlled Pilot Trial. Int J Sports Med. 2006;27(7):573-580.
53. Schmitz KH, Ahmed RL, Hannan PJ, Yee D. Safety and Efficacy of Weight Training in Recent Breast Cancer Survivors to Alter Body Composition, Insulin, and Insulin-Like Growth Factor Axis Proteins. J Cancer Epidemiol Prev. 2005;14(7):1672-1680.
54. Kim C, Kang D-H, Park J-W. A Meta-Analysis of Aerobic Exercise Interventions for Women With Breast Cancer. Western Journal of Nursing Research. 2009;31(4):437-461.
55. Courneya KS, Mackey JR, Bell GJ, Jones LW, Field CJ, Fairey AS. Randomized Controlled Trial of Exercise Training in Postmenopausal Breast Cancer Survivors: Cardiopulmonary and Quality of Life Outcomes. J Clin Oncol. May 1, 2003 2003;21(9):1660-1668.
56. Newman AB, Kupelian V, Visser M, et al. Strength, But Not Muscle Mass, Is Associated With Mortality in the Health, Aging and Body Composition Study Cohort. The Journals of Gerontology Series A: Biological Sciences and Medical Sciences. January 1, 2006 2006;61(1):72-77.
57. Williams T. Physical fitness and activity as separate heart disease risk factors: a meta-analysis. Medicine & Science in Sports and Exercise. 2001;33(5):754-761.
58. Loprinzi CL, Athmann LM, Kardinal CG, et al. Randomized Trial of Dietician Counseling to Try to Prevent Weight Gain Associated with Breast Cancer Adjuvant Chemotherapy. Oncology. 1996;53(3):228-232.
59. Jen KLC, Djuric Z, DiLaura NM, et al. Improvement of Metabolism among Obese Breast Cancer Survivors in Differing Weight Loss Regimens[ast][ast]. Obesity. 2004;12(2):306-312.
60. Thompson CATATSJWBECENGFPA, 1152 P. Changes in Body Weight and Metabolic Indexes in Overweight Breast Cancer Survivors Enrolled in a Randomized Trial of Low-Fat vs. Reduced Carbohydrate Diets Nutrition and Cancer. 2010;62(9):1142-1152.
61. Shaw C, Mortimer P, Judd PA. A randomised controlled trial of weight reduction as a treatment for breast cancer-related lympedema. Cancer. 2007;110:1868-1874.
62. Chlebowski RT, Blackburn GL, Thomson CA, et al. Dietary Fat Reduction and Breast Cancer Outcome: Interim Efficacy Results From the Women's Intervention Nutrition Study. J. Natl. Cancer Inst. December 20, 2006 2006;98(24):1767-1776.
63. Saquib N, Natarajan L, Rock CL, et al. The impact of a long-term reduction in dietary energy on body weight within a randomized diet trial. Nutrition and Cancer. 2008;60(1):31-38.
64. Bryner RW, Ullrich IH, Sauers J, et al. Effects of Resistance vs. Aerobic Training Combined With an 800 Calorie Liquid Diet on Lean Body Mass and Resting Metabolic Rate. J Am Coll Nutr. April 1, 1999 1999;18(2):115-121.
65. Stiegler P, Cunliffe A. The Role of Diet and Exercise for the Maintenance of Fat-Free Mass and Resting Metabolic Rate During Weight Loss. Sports Medicine. 2006;36:239-262.
66. Mefferd K, Nichols JF, Pakiz B, Rock CL. A cognitive behavioral therapy intervention to promote weight loss improves body composition and blood lipid profiles among overweight breast cancer survivors. Breast Cancer Research and Treatment. 2007;104:145-152.
67. Demark-Wahnefried W, Case LD, Blackwell K, et al. Results of a Diet/Exercise Feasibility Trial to Prevent Adverse Body Composition Change in Breast Cancer Patients on Adjuvant Chemotherapy. Clinical Breast Cancer. 2008;8(1):70-79.
68. 68. Morey MC, Snyder DC, Sloane R, et al. Effects of Home-Based Diet and Exercise on Functional Outcomes Among Older, Overweight Long-term Cancer Survivors: RENEW: A Randomized Controlled Trial. JAMA. May 13, 2009 2009;301(18):1883-1891.
69. Demark-Wahnefried W, Clipp EC, Lipkus IM, et al. Main Outcomes of the FRESH START Trial: A Sequentially Tailored, Diet and Exercise Mailed Print Intervention Among Breast and Prostate Cancer Survivors. J Clin Oncol. July 1, 2007 2007;25(19):2709-2718.
70. Buckley JD, Howe PRC. Anti-obesity effects of long-chain omega-3 polyunsaturated fatty acids. Obesity Reviews. 2009;10(6):648-659.
71. Hill A, Buckley JD, Murphy KJ, Howe PRC. Combining fish-oil supplements with regular aerobic exercise improves body composition and cardiovascular disease risk factors. Am J Clin Nutr. 2007;85:1267-1274.
72. Kabir M, Skurnik G, Naour N, et al. Treatment for 2 mo with n 3 polyunsaturated fatty acids reduces adiposity and some atherogenic factors but does not improve insulin sensitivity in women with type 2 diabetes: a randomized controlled study. Am J Clin Nutr. December 1, 2007 2007;86(6):1670-1679.
73. Colomer R, Moreno-Nogueira JM, García-Luna PP, et al. n-3 Fatty acids, cancer and cachexia: a systematic review of the literature. Br J Nutr. 2007;97(05):823-831.
74. Calder PC. Polyunsaturated fatty acids and inflammatory processes: New twists in an old tale. Biochimie. 2009;91(6):791-795.
75. Mishra A, Chaudhary A, Sethi S. Oxidized Omega-3 Fatty Acids Inhibit NF-{kappa}B Activation Via a PPAR{alpha}-Dependent Pathway. Arterioscler Thromb Vasc Biol. September 1, 2004 2004;24(9):1621-1627.
76. Babcock TA, Kurland A, Helton WS, Rahman A, Anwar KN, Espat NJ. Inhibition of activator protein-1 transcription factor activation by omega-3 fatty acid modulation of mitogen-activated protein kinase signaling kinases. JPEN J Parenter Enteral Nutr. 2003;27(3):176-180.
77. Volpi E, Ferrando AA, Yeckel CW, Tipton KD, Wolfe RR. Exogenous amino acids stimulate net muscle protein synthesis in the elderly. Journal of Clinical Investigations. 1998;101:2000-2007.
78. Aquilani R, Opasich C, Gualco A, et al. Adequate energy-protein intake is not enough to improve nutritional and metabolic status in muscle-depleted patients with chronic heart failure. Eur J Heart Fail. 2008;10:1127-1135.
79. Dillon EL, Sheffield-Moore M, Paddon-Jones D, et al. Amino Acid Supplementation Increases Lean Body Mass, Basal Muscle Protein Synthesis, and Insulin-Like Growth Factor-I Expression in Older Women. J Clin Endocrinol Metab. May 1, 2009 2009;94(5):1630-1637.

2.4 Omega-3 fatty acids (LCn-3): Function and influences on body composition

2.4.0 Overview of LCn-3 in the context of inflammation and body composition change

LBM maintenance may be partly controlled by the balance of inflammation in the body (Mitch and Goldberg 1996). In healthy active populations, the acute inflammatory response as a result of physical activity triggers a cascade of pathways that effectively remove damaged muscle tissue and initiate growth and repair resulting in net LBM gains. The inflammatory response is responsible for break down and removal of damaged tissues, and signals growth factors to the site so that growth can begin (Ploeger et al. 2009). However, low-grade chronic inflammation often identified by slightly but consistently higher than baseline levels of inflammatory cytokines (Interleukin-6 and C-Reactive Protein)(Jensen 2008) may be responsible for greater muscle tissue breakdown with a dampened anabolic or growth response. This is turn may lead to inflammation related myopenia, which is related to numerous functional and metabolic sequelae (Fearon, Evans, and Anker 2011). In breast cancer populations, chronic low grade inflammation as measured by CRP and serum amyloid A (SAA) was predictive of survival, however the relationship of LBM change was not addressed (Pierce, Ballard-Barbash, et al. 2009). Apart from physical activity, certain nutrients have been shown to exert anti-inflammatory effects that may decrease chronic inflammation and be protective of LBM (Jensen 2008). LCn-3s are nutrients that have a sound theoretical, rodent and human literature base that support the potential in ameliorating sequelae of chronic inflammation(Calder 2012).

This section will give a background of the rationale and mechanisms for the anti-inflammatory action of LCn-3s. The second part of this section is a published paper that explores the evidence for LCn-3 supplementation and it's effect on absolute and functional LBM. In addition, the published paper also gives insight into dosing and tissue uptake of LCn-3s in humans that is likely to influence results observed in the published literature.

2.4.1 Rationale and overview of LCn-3, inflammation and body composition

Omega-3 FA, inflammation and body composition

Severe acute and low-grade chronic inflammation, via the ubiquitin-proteasome pathway, can lead to proteolysis and net LBM loss (Mitch and Goldberg 1996). Ongoing research continues to confirm that LCn-3s are critical for appropriate inflammatory response and cessation (Calder 2012). In addition, LCn-3s are thought to influence fuel utilization in the mitochondria resulting in higher fat oxidation in humans (Buckley and Howe 2010). Taken together, LCn-3s present as potential therapeutic agents in maintaining LBM and helping to reduce body fat mass. This section details

some of the proposed and known mechanisms that explain LCn-3s role in maintaining a healthy body composition.

LCn-3s, inflammation and LBM

The acute inflammatory response is necessary in order for the body to defend itself and to heal. However, it is generally agreed that cardiovascular disease and other conditions related to metabolic syndrome are largely driven by chronic low-grade inflammation (Egger and Dixon 2009). Similarly, in diseases such as chronic heart failure, chronic obstructive pulmonary diseases and cancer, prolonged higher concentration of inflammatory cytokines and signaling proteins are related to disease progression and certain co-morbidities, in particular myopenia, or muscle wasting (Fearon, Evans, and Anker 2011). A considerable amount of research has investigated the roles of diet and physical activity in reducing chronic inflammation. Over the past two decades LCn-3s have been extensively researched to determine the function and effect of their anti-inflammatory action. LCn-3s are thought to influence a number of pathways associated with the stimulation and propagation of the inflammatory response (Calder 2012). These include relationships with leucocyte chemotaxis, reduced adhesion molecule expression and decreased leucocyte-endothelium interaction, decreased AA derived eicosanoid production and AA containing endocannabinoids, increased production of weak EPA eicosanoids and EPA and DHA containing endocannabinoids, increased production of pre-resolutions resolvins and protectins, decreased production of inflammatory cytokines, decreased T-cell reactivity (Calder 2012). Considering the potential role of inflammation in LBM decline, elucidating the role of LCn-3s may enable better management of myopenia in populations that are susceptible. Relevant pathways to LBM protection are discussed below.

LCn-3s and their effects on inflammatory mediators

Overall, higher concentrations of LCn-3s in the diet are likely to promote a dampening of the pro-inflammatory response through a number of pathways (Wada et al. 2007, Fetterman Jr and Zdanowicz 2009). LCn-3s competitively inhibit the production of more pro-inflammatory AA derived eicosanoids, and independently of eicosanoid production (Fetterman Jr and Zdanowicz 2009). LCn-3s directly inhibit the activation of Nuclear Factor kappa B (Nf-kB) (Boutros et al. 2010, Calder 2012) in part through agonistic ligand binding of PPAR-Gamma complexes, which in turn reduces transcription of TNF-a, IL-1 and IL-6 mRNAs (Flachs et al. 2009). Many of these effects may be due to, and/or in addition to, their emerging role in cell membrane lipid rafts (Singer 2004, Chapkin et al. 2009). These functions become relevant to homeostasis of muscle protein, as all of these inflammatory pathways up-regulate the ubiquitin-proteasome pathway (UPP), which when activated increases proteolysis (Smith, Mukerji, and Tisdale 2005, Wyke et al. 2005,

Simmons et al. 1984). Proteolysis is normal in healthy populations, however it is typically balanced by protein synthesis. In acute or chronic inflammation higher rates of proteolysis are observed, and is amplified by an inhibition of protein synthesis (Mitch and Goldberg 1996). Taken together, LCn-3s affect many of the pathways associated with proteolysis, and have a theoretical role in decreasing LBM breakdown related to increased inflammation.

LCn-3 and their role in fat oxidation

LCn-3 may reduce body fat by up-regulating genes associated with fatty acid oxidation (Buckley and Howe 2010). A number of human trials have shown that LCn-3 supplementation resulted in reduction of body weight (Kabir et al. 2007), body fat (Hill et al. 2007, Noreen et al. 2010, Couet et al. 1997), and adipocyte diameter (Kabir et al. 2007). In addition, a small but well controlled study reported an increase of fatty acid oxidation after LCn-3 was increased in their meals for 3 weeks (Couet et al. 1997). In animal and in-vitro studies, LCn-3s have been shown to increase transfer of acyl groups into the mitochondria for beta-oxidation by up regulating the expression of mitochondrial Carnitine palmitoyl transferase I (CPT-I) in visceral/ectopic fat, but not subcutaneous fat (Flachs et al. 2005). Visceral/ectopic fat deposition has been shown to be more indicative of metabolic disease and inflammation when compared to subcutaneous fat. In addition, it is thought that LCn-3s up-regulate the production of uncoupling protein (UCP)-3 in skeletal muscle mitochondrial cell membranes (Hun Cha et al. 2001). UCP-3 causes mitochondrial proton leakage, which reduces ATP production and increases heat output, thus more fatty acids are needed for the same ATP output. In conjunction with this, LCn-3s are also thought to increase peroxisome acyl CoA oxidase (Acyl-CoA) (Flachs et al. 2009). Peroxisome fatty acid oxidation, which is increased with presence of Acyl-CoA is less efficient than mitochondrial beta-oxidation yielding 30-40% less ATP and 30% more heat. Taking the in-vitro findings for these three mechanisms, LCn-3s may increase resting fatty acid oxidation particularly in visceral/ectopic fat tissue, which could result in a loss of fat mass and/or a reduction in metabolic dysfunction.

2.5 Published Manuscript #2

LCn-3 and their role in body composition change

This published review summarises previous studies that have investigated the role of LCn-3s in body composition change in humans. It outlines the available evidence for their efficacy in humans and reviews the dosage strategies required to maximize the effect of LCn-3s in trials. An important finding of this review relates to the effects of LCn-3 in conjunction with exercise. A number of trials over the last four years have investigated elements of LBM function and response, however to date there has been no compilation of these findings. Additionally, LCn-3 studies are notoriously heterogeneous when it comes to dosing and measurement of dosing adherence, thus the review addresses methodological considerations for this as well.

NRC Research Press

REVIEW

Omega-3 fatty acids and changes in LBM: alone or in synergy for better muscle health?[1]

Cameron McDonald, Judy Bauer, and Sandra Capra

Abstract: Myopenia or muscle wasting due to ageing, chronic disease, and various medical interventions has been associated with increased mortality, morbidity, and poorer physical function. Attempts through nutrient and exercise interventions have been made to prevent this deterioration. In addition, while a measure of lean body mass (LBM) is associated with health outcomes, LBM function may be a better prognostic tool. Long-chain omega-3 fatty acids (LCn-3s) are nutrients that may mitigate LBM losses in noncancer populations. The purpose of this review is to determine whether LCn-3s have a role in LBM sparing in noncancer populations, to establish a minimum dose and duration of LCn-3s that will result in LBM change, and to summarise the potential effects of LCn-3s on LBM function when combined with an anabolic stimulus. Overall, in noncancer populations, LCn-3s have limited utility in sparing LBM during energy balance, energy restriction, or in conjunction with aerobic exercise. Further investigations are required to determine the appropriate dose and duration of LCn-3s for optimal LBM function. Finally, compelling evidence exists for LCn-3s in conjunction with an anabolic stimulus to improve LBM function and quality. Functionality of LBM tissue is an important outcome for population health, and LCn-3s show some promise, albeit pending further study.

Key words: omega-3 fatty acids, lean body mass, myopenia, sarcopenia, exercise, physical activity.

Résumé : La myopénie ou perte musculaire due au vieillissement, aux maladies chroniques et à différentes interventions médicales a été associée à un accroissement de mortalité, de morbidité et à un appauvrissement de la fonction physique. Des tentatives d'interventions nutritionnelles et d'exercices ont été faites pour prévenir cette détérioration. En outre, alors que la mesure de la masse maigre est associée des résultats cliniques, la fonction de la masse maigre pourrait constituer un meilleur outil pronostique. Les acides gras oméga-3 à longue chaine (LCn-3) sont des nutriments qui peuvent atténuer la perte musculaire chez des populations non atteintes de cancer. Le but de cet article de synthèse est de déterminer si les LCn-3 jouent un rôle dans la préservation de la masse maigre chez des populations non atteintes de cancer, d'établir une dose minimale de LCn-3 et une durée de traitement minimale qui résulteraient en un changement de la masse maigre, ainsi que de résumer les effets potentiels des LCn-3 sur la fonction de la masse maigre lorsque combinés à un stimulus anabolique. Dans l'ensemble, chez des populations non atteintes de cancer, les LCn-3 ont une utilité limitée dans la préservation de la masse maigre en condition de balance énergétique, de restriction énergétique ou en combinaison avec un exercice aérobique. Des recherches plus poussées sont nécessaires pour déterminer la dose appropriée et la durée d'un traitement aux LCn-3 pour maintenir une fonction optimale de la masse maigre. Finalement, il existe une preuve convaincante que les LCn-3 combinés à un stimulus anaérobique améliorent la fonction et la qualité de la masse maigre. Une masse maigre fonctionnelle est un résultat clinique important en santé des populations et les LCn-3 sont prometteurs, bien que des études plus poussées soient requises. [Traduit par la Rédaction]

Mots-clés : acides gras oméga 3, masse maigre, myopénie, sarcopénie, exercice, activité physique.

Introduction

Skeletal muscle mass, commonly referred to as lean body mass (LBM), is the major reservoir of amino acids in the body, accounting for 50%–60% of the body's stores (Lenk et al. 2010; Muscaritoli et al. 2010). Its primary purposes are to provide movement, strength, and respiration (Lenk et al. 2010), with recent evidence indicating that skeletal muscle mass has important functions in immunosufficiency (Castaneda et al. 1995; Roubenoff 2008). However, because of a number of behavioural and disease factors, muscle wasting, recently described using the term myopenia (Fearon et al. 2011*a*; 2011*b*), has become common and is becoming established as an important prognostic marker of disease and general health. The term myopenia is preferred to others because it can represent all types of muscle wasting, and it translates well in other languages (Fearon et al. 2011*a*). Sarcopenia is a term that has been primarily used to describe clinically significant muscle wasting in the elderly, while cachexia is defined as "… a multifactorial syndrome defined by an ongoing loss of skeletal muscle mass that cannot be fully reversed by conventional nutrition support …" (Fearon et al. 2011*b*), recognised in association with a number of conditions. However, muscle wasting is found at the centre of both sarcopenia and cachexia, and therefore the term myopenia is appropriate as a general classification (Fearon et al. 2011*a*).

Myopenia is defined as "… a clinically relevant degree of muscle wasting that is associated either with impaired functional capacity and (or) with increased risk of morbidity or mortality" (Fearon et al. 2011*a*). It has been estimated to affect 20% of those aged over 60 years (Taaffe 2006), and is associated with disuse, malnutrition, acute and chronic inflammation, and cachexia (Muscaritoli et al. 2010; Fearon et al. 2011*a*; 2011*b*). Muscle wasting is directly associated with loss of strength, increased likelihood of falls, and loss of autonomy. It is also associated with increased mortality in healthy older populations (Stenholm et al. 2008; Bunout et al. 2011; Gen-

Received 3 September 2012. Accepted 9 November 2012.

C. McDonald, J. Bauer, and S. Capra. Centre for Dietetics Research, Level 5, Human Movement Studies building, University of Queensland, Brisbane, Australia.
Corresponding author: Cameron McDonald (e-mail: c.mcdonald4@uq.edu.au).

[1]*This review is one of a number of papers published in the Special Issue on Food as Medicine.*

ton et al. 2012). Furthermore, evidence from large epidemiological studies indicates that the loss of muscle function may be more indicative of mortality and adverse health outcomes than one's absolute loss of LBM (Newman et al. 2006; Ruiz et al. 2008). Myopenia is also associated with ectopic deposition of intramuscular triglyceride, which has been associated with chronic inflammation, increased insulin resistance (Goodpaster et al. 2000), and greater incidence of cardiovascular disease (Hamdy et al. 2006).

Studies that aimed to determine the mechanistic aspects of myopenia have reported that acute and chronic inflammation are associated with both increased muscle proteolysis and reduced response to anabolic stimuli, also known as anabolic resistance (Breen and Phillips 2011; Haran et al. 2012). A wealth of literature indicates that both aerobic and resistance exercises increase LBM function, while resistance training alone is regarded as the intervention associated with the most improved strength and size of the LBM. In young healthy, elderly, and diseased populations, studies have investigated strategies to prevent myopenia and to ameliorate anabolic resistance with the aim of enhancing the muscle protein synthetic (MPS) response to an anabolic stimulus, such as resistance exercise. Typically, the interventions of interest have targeted antiinflammatory (Cerchietti et al. 2007; Murphy et al. 2011*a*; 2011*b*), anabolic agents (Breen and Phillips 2011; Deutz et al. 2011), or combinations of both (Rogers et al. 2011) in an attempt to mitigate these changes in muscle mass and function. Long-chain omega-3 fatty acids (LCn-3s) have been proposed to preserve LBM through their antiinflammatory action and subsequent downregulation of the ubiquitin–proteasome pathway (Calder 2009).

Over the last 2 decades, LCn-3s have become a common dietary supplement. Initially, LCn-3s were promoted as an effective adjunct in secondary prevention of cardiovascular disease (Wang et al. 2006) and over time have been used in many other conditions for which there are acute and chronic inflammatory backgrounds. One area of investigation in response to the rise in obesity is the potential for LCn-3s to positively influence body composition. However, recent systematic reviews addressing some of these issues indicate that little to no evidence exists to promote LCn-3s for body weight loss (Martínez-Victoria and Yago 2012), while some evidence exists for its use in reducing body fat in lieu of weight change (Buckley and Howe 2009). However, from all of the trials reviewed, the magnitude of change in body fat may have minimal clinical utility (Buckley and Howe 2009).

LCn-3s have been established as anti-inflammatory agents in humans, and the mechanisms of their action have been extensively reviewed elsewhere. In brief, LCn-3s offer protection from proteolysis and subsequent muscle wasting, through altered genetic expression and a variety of immunomodulatory pathways (Babcock et al. 2003; Novak et al. 2003; Mishra et al. 2004; Singer 2004; Edwards and O'Flaherty 2008; Calder 2009; Chapkin et al. 2009). While acute inflammation is normal, the presence of major acute or low grade chronic inflammation leads to higher rates of proteolysis, and is also seen to inhibit protein anabolism (Haran et al. 2012). With regards to the deposition of ectopic or intramuscular triglyceride, LCn-3s are thought to decrease this through enhanced mitochondrial lipid oxidation via some pathways including upregulation of uncoupling protein-3 (Hun Cha et al. 2001; Buckley and Howe 2009) and carnitine palmitoyl transferase (Buckley and Howe 2010). In contrast, the long-chain omega-6 fatty acid linolenic acid (LA), which is the precursor for arachadonic acid, is typically responsible for the production of proinflammatory eicosanoids (Calder 2009). The content of LA in the Western diet is much higher than LCn-3s, i.e., LCn-6:LCn-3 has been reported as 15–20:1, and there is some evidence indicating a negative effect of this unbalanced ratio that is thought to be optimal at 1–4:1 (Simopoulos 1999).

The proposed mechanisms of LBM preservation and increased fatty acid oxidation lead to the hypothesis that LCn-3s may improve body composition. On the other hand, epidemiological evidence that better survival for individuals is associated with more functional LBM, rather than higher LBM alone, suggests that management should include a measure of muscle function to validate an agent's efficacy (Newman et al. 2006; Ruiz et al. 2008). In regards to the potential of LCn-3s to assist in maintenance or improvement of muscle function, little discussion has taken place in published literature.

Therefore, the purpose of this review is to answer the following questions: (*i*) Do LCn-3s have an effect on change in LBM when taken alone or in conjunction with energy restriction or an exercise program? (*ii*) If there is an effect, what is the minimum dose of LCn-3s required to elicit change in LBM, or LBM protection, and what factors influence the objective measurement of LCn-3s with relevance to LBM change? (*iii*) Does the addition of LCn-3s to an anabolic stimulus (e.g., an amino acid dose or exercise program) have a synergistic effect on LBM or LBM function?

Materials and methods

A systematic search was carried out using the MEDLINE and Pubmed databases. Key terms used included omega 3 fatty acids (n-3 fatty acids, omega 3, and polyunsaturated fatty acids), body composition (percentage body fat, muscle mass, LBM, and skeletal muscle), exercise (physical activity, resistance training, and aerobic training), tissue uptake (cholesterol esters, phospholipids, serum lipids, erythrocytes, and adipose tissue), LBM quality, and LBM function (muscle strength, intramuscular triglycerides, muscle protein synthesis, anabolic resistance, peak torque, and mitochondrial expansion). With regards to LCn-3 supplementation and LBM change, trials were included if they reported on an adult (>18 years of age) noncancer or noncachectic population, used fatty fish or LCn-3 supplementation as capsules, emulsion, or other, had a concurrent control group (randomised or pseudo-randomised), and reported on changes in LBM. Studies and reviews of studies with the primary objective of assessing dose–response and subsequent tissue uptake of LCn-3s were included if they or the studies they reviewed reported an objective measure of LCn-3 content in at least one bodily tissue before and after supplementation taken as capsules or emulsions. Studies reporting the effects of LCn-3s in conjunction with an anabolic stimulus on LBM function were included if they reported an objective measure of LCn-3s and an objective measure of LBM function or quality before and after supplementation with LCn-3s, were uncontrolled or controlled trials, and included adults (>18 years of age) from any population. The quality of the studies was assessed using the Academy of Nutrition and Dietetics's Evidence Analysis handbook. Assessment of the studies included grading studies by research design (Class A through to D) and quality criteria (Positive, Neutral, and Negative), giving a study an overall rating of high, neutral, or poor (Academy of Nutrition and Dietetics 2010).

Do LCn-3s have an effect on change in LBM when taken alone or in conjunction with energy restriction or an exercise program?

To date, there have been 10 controlled trials that have assessed the effect of LCn-3s on change in LBM over time (Table 1). Three of these studies have investigated the effects of LCn-3s alone while maintaining energy balance through controlled energy intake or normal intake (Couet et al. 1997; Noreen et al. 2010; Crochemore et al. 2012), 6 studies have reported the effects of LCn-3s on LBM during weight loss (Storlien et al. 2001; Krebs et al. 2006; Thorsdottir et al. 2007; Abete et al. 2008; Hlavaty et al. 2008; Munro and Garg 2012), and 1 study has combined aerobic exercise with or without LCn-3 supplementation (Hill et al. 2007). Overall, there was significant heterogeneity among studies in regards to LCn-3 dose, length of intervention, prescribed energy deficit, and control of LCn-3 adherence. In addition to this, the quality of the papers have been generally less than optimal in that of the 10

Table 1. Controlled trials assessing the impact of LCn-3s on lean body mass.

Reference study and quality rating	Population	Measures	Dose, duration, intervention, and measures	Reported change in LBM	Comments
Couet et al. 1997; non-RCT cross-over time series; quality, +ve	*n* = 6; young healthy adults; mean BMI, 21.9±1.6 kg/m^2; mean body fat, 12.42%	Body composition, DEXA; adherence, LCn-3 content of platelet phospholipids; PA, assessed; diet, assessed	INT, capsules (EPA, 1.1 g/day and DHA, 0.7 g/day); control, non-n3FA oil added in; 3 weeks control diet; 10–12 week washout; and 3 weeks intervention (FO)	INT, +0.2 kg; control, –0.24 kg; other body composition; NS ($p > 0.05$)	Strengths: total control of diet during intervention. High quality measure cross-over design. Limitations: short duration
Noreen et al. 2010; 2-arm RCT; quality, —	*n* = 44; age, 18–55 years; healthy, no metabolic/ cardiac disease	Body composition, BodPod (ADP); adherence, not reported; PA, not measured; diet, not measured	INT, capsules (EPA, 1.6 g/day and DHA, 0.8 g/day); control, 4 g/day sunflower oil; 6 weeks supplementation + maintain current habits	INT, +0.5 kg (95% CI 0.3–0.8); control, –0.1 kg (95% CI –0.6–0.4); $p = 0.03$	Strengths: high dose LCn-3s and quality of assessment. Limitations: no diet or physical activity reported and no adherence of pills reported
Crochemore et al. 2012; 3-arm RCT; Quality, –ve	*n* = 41; age 60.78 years; T2DM, menopausal, and MetX risk factors	Body composition, BIA; adherence, pill count; PA, not measured; diet, not measured	INT-high, capsules (EPA, 0.55 g and DHA, 0.35 g); INT-low (EPA, 0.32 g and DHA, 0.21 g); and control, gelatin + vitamin E; 30 days with no other conditions reported	INT-high, +0.66%; INT-low, +0.38%; control, +1.1%; NS within groups and NS among groups	Limitations: low dose, short duration, poor quality body composition measure, no control of diet or PA, and no measure of pill adherence
LCn-3s in conjunction with prescribed energy restriction					
Thorsdottir et al. 2007; 4-arm RCT; quality, +ve	*n* = 324 → 278 final (no difference in dropouts); age, 20–40 years; WC, >94 cm men and >80 cm women; BMI, 27.5–32.5 kg/m^2	Body composition, BIA; adherence; diet, assessed; PA, assessed	Total EPA/DHA content: INT, 450 g lean fish/week (0.3 g/day); INT, 450 g fatty fish/week (3 g/day); INT, capsules (1.5 g/day); control, no seafood + placebo; 8 week intervention, tailored 30% dietary energy restriction	No significant change reported in LBM for any group. LBM values not shown	Large sample size and variation in LCn-3 delivery. Significant drop out and poor quality body composition measurements
Abete et al. 2008; 2-arm pseudo-RCT; unblinded; quality, –ve	*n*=40; *n* = 32; male and female; age, 36 years (7); BMI, 31.6 kg/m^2 (3.5); otherwise healthy	Body composition, BIA; adherence, not reported; diet, assessed; PA, assessed	INT, 3 fatty fish/week (EPA, 0.15 g and DHA, 0.42 g); control, no fish, ALA LCn-3 only; 8 weeks plus 30% energy restriction (30% fat, 53% CHO, and 17% protein)	INT, –2.4 kg; control, –2.4 kg; $p = 0.983$ between groups	Controlled for diet. Low dose, poor quality measure, and low protein content of diet may account for ++LBM loss
Hlavaty et al. 2008; 2-arm RCT; blinding unknown; quality, –ve	*n* = 40 (100% female); age, N3, 55.2 years (13.2); control, 59 years (10.2); moderately obese; BMI, N3, 33.1 kg/m^2; control, 36.2 kg/m^2	Body composition, BIA; adherence, serum triglycerides analysis; diet, all food supplied; PA, not reported	INT, yoghurt enriched with LCn-3s (620 mg EPA+DHA, individual not given); control, yoghurt with no LCn-3s; phase 1, 3 days, weight stabilisation, and inpatient; phase 2, 21 days inpatient, 2500 kJ/day restriction + 60 min; PA/day (not defined), controlled meal production	INT, –0.8 kg; control, +1.8 kg; $p < 0.008$ favouring control	Differences in initial weight confounded all results. Low dose LCn-3s used. Low quality body composition measure; control significantly more obese at bline for weight, body fat %, fat mass (kg), and LBM (kg)

Table 1 (*concluded*).

Reference study and quality rating	Population	Measures	Dose, duration, intervention, and measures	Reported change in LBM	Comments
Krebs et al. 2006; 3-arm RCT; quality, +ve	n = 116; completed, n = 93 (100% female); age, 44.7 years (21–69); BMI, 35 kg/m^2 (range: 26.2–47.6); 100% insulin resistant	Body composition, DEXA + CT scan (L2–L4); adherence, plasma and adipose fatty acids; diet, not reported; PA, not reported	INT, capsules (EPA, 1.3 g and DHA, 2.9 g); placebo, capsules (LA, 2.8 g and oleic acid, 1.4 g); control, none; 24 week intervention, 3.3–3.8 MJ/day (MR) for 5 weeks, reintroduced food 7 weeks, weight maintenance 12–24 weeks; INT, LCn-3+energy restriction; placebo, energy restriction alone; C–control, no LCn-3s or energy restriction	No change in LBM for any group. Results not shown	High dose prescribed. Effective weight loss program. Physical activity not recorded. Milk-based diet may protect lean mass (high protein and calcium)
Storlien et al. 2001; 3-arm RCT; open label; quality: —	n = 52 (34 females); age: female, 48 years and male, 46 years; mild to moderate HTN; obese, 130%–170% IBW	Body composition, UWW; adherence, not reported; diet, food provided by study; PA, measured, not shown (no changes reported)	Total EPA/DHA content unknown; phase 1, 7 day inpatient isocaloric; phase 2, 10 week energy deficit (males, 1800 kcal/day and females, 1200 kcal/day); with differing polyunsaturated:saturated (P:S) fat content; INT, P:S = 1.0 (LCn-3s 25% of fat); n-6FAs, P:S = 1.0 (n-6FAs 25% of fat); control, P:S = 0.25	INT, –1.23 kg; n-6FA, –2.92 kg; control, –2.68 kg; $p > 0.05$ (NS); and % body weight lost as LBM: INT, 14.3%; n-6FA, 28.6%; control, 24% (NS)	No defined levels of LCn-3. Diet controlled and high quality measures used. Trend for less muscle loss for omega-3 group. Not sufficiently powered for a 5% change. Differences in baseline weight between groups
Munro and Garg 2012; 2-arm RCT; quality, —	n = 40; 32 final; BMI, 33 kg/m^2; otherwise healthy	Body composition, DEXA; adherence, RBC fatty acids; diet, measured; PA, not reported	INT, capsules (EPA, 0.42 g and DHA, 1.62 g); control, sunola oil; 4 weeks weight loss (3000 kJ/day), then 10 weeks body weight maintenance	INT, –1.36 kg; control, –1 kg; $p > 0.05$ (NS)	High DHA dose, high quality body composition measure, and correlation found between fat lost and EPA and DHA. No measure of physical activity
Hill et al. 2007; 4-arm RCT; double blind; quality, +ve	n = 81; 75 final (28 men); age, 52; body fat %, 43.9 + at least 1 MetX RF	Body composition, DEXA; adherence, RBC fatty acids; diet, measured; PA, compliance (>85%)	LCn-3 groups, (EPA, 0.36 g and DHA, 1.56 g); placebo, sunflower oil, 6 g; 2 × 2 factorial, n–3, placebo + exercise, no exercise; 12 weeks exercise, run/walk, 45 min, 3 sessions/week at 75% age-predicted maximum heart rate	Data not shown. Stated that LBM did not differ between groups	High dose of DHA and high quality body composition measures. Good measures of compliance

Note: LCn-3s, long-chain omega-3 fatty acids; LBM, lean body mass; RCT, randomised control trial; BMI, body mass index; DEXA, dual-energy X-ray absorptiometry; PA, physical activity; INT, intervention; EPA, eicosapentanoic acid; DHA, docosapentanoic acid; FO, fish oil; NS, nonsignficant; ALA, α-linolenic acid; ADP, air displacement plethysmography; T2DM, type II diabetes mellitus; MetX RF, metabolic syndrome X risk factors; BIA, bioelectrical impedance; diet, dietary intake; WC, waist circumference; BMI, body mass index; HTN, hypertension; IBW, ideal body weight; UWW, underwater weighing; n-6FAs, long-chain omega-6 fatty acids; RBC, red blood cells; +ve, high quality; —, neutral quality; and –ve, poor quality

studies (Table 1), only 4 were allocated a rating of high (Couet et al. 1997; Krebs et al. 2006; Hill et al. 2007; Thorsdottir et al. 2007), 3 were neutral (Storlien et al. 2001; Noreen et al. 2010 Munro and Garg 2012), and 3 were poor (Abete et al. 2008; Hlavaty et al. 2008; Crochemore et al. 2012).

LCn-3s alone and changes in LBM

Three studies have investigated the influence of LCn-3s on change in LBM during energy balance, i.e., stable energy intake aiming for weight stability, in healthy individuals. Two studies used a high quality measure of body composition, dual-energy X-ray absorptiometry (DEXA) (Couet et al. 1997) and air displacement plethysmography (ADP) (Noreen et al. 2010), respectively, while one used bioelectrical impedance (Crochemore et al. 2012). Duration of the studies was not consistent, with 3 week (Couet et al. 1997), 30 day (Crochemore et al. 2012), and 6 week (Noreen et al. 2010) intervention periods. Similarly, LCn-3 supplementation varied from 0.53 to 2.4 g/day. Only 1 study reported an objective measure of LCn-3 tissue uptake, and the same study was the only 1 of the 3 to appropriately account for dietary intake and physical activity during the study period (Couet et al. 1997).

In regards to LBM change, Noreen et al. (2010) reported a statistically significant increase in LBM (+0.5 ± 0.5 kg) when compared with the control (−0.1 ± 1.2 kg) after 6 weeks of supplementation with 1.6 g eicosapentanoic acid (EPA) and 0.8 g docosahexanoic acid (DHA) in healthy middle-aged men and women. Couet et al. (1997) reported a trend for LBM to increase after 3 weeks of supplementation (1.1 g EPA and 0.7 g DHA); however, it did not reach statistical significance. Crochemore et al. (2012) reported no difference in LBM change after 30 days of varying, albeit, lower daily doses of LCn-3 (lower dose, 0.33 g EPA and 0.21 g DHA; higher dose, 0.55 g EPA and 0.35 g DHA). Taken together, these results, while inconclusive, indicate that higher doses (>1.7 g LCn-3) for longer durations (>6 weeks) may be required to realise benefit for LBM. Regardless of this, even the greater change in LBM only reached 0.5 kg (Noreen et al. 2010), which may have limited clinical significance and is within the error ranges of the devices used to estimate body composition.

Controlled trials of LCn-3s plus energy restriction and changes in LBM

Of the 6 trials that reported LBM changes after energy restriction with or without LCn-3 supplementation, 1 study showed a trend for LBM retention during weight loss (Storlien et al. 2001), while the other 5 showed no effect (Krebs et al. 2006; Thorsdottir et al. 2007; Abete et al. 2008; Hlavaty et al. 2008; Munro and Garg 2012).

All 6 studies were designed to determine whether LCn-3s enhanced weight loss in conjunction with energy restriction. Again, heterogeneity existed in regards to dosage of LCn-3, duration, type and amount of energy restriction, and measurement of body composition. Only 2 of the studies were assessed as high quality (Krebs et al. 2006; Thorsdottir et al. 2007), 2 were considered of neutral quality (Storlien et al. 2001; Munro and Garg 2012), and 2 were of poor quality (Abete et al. 2008; Hlavaty et al. 2008).

High quality measures of body composition, DEXA (Krebs et al. 2006) and underwater weighing (Storlien et al. 2001), were used in 2 studies, while bioelectrical impedance analysis (BIA) was used for the remaining 4 (Thorsdottir et al. 2007; Abete et al. 2008; Hlavaty et al. 2008; Munro and Garg 2012). Prescribed daily LCn-3 intake was unknown in 1 study using capsules (Storlien et al. 2001) and ranged from 0.57 to 4.2 g/day in the remaining 5 studies. An objective measure of adherence (tissue content of LCn-3s) was assessed in 3 studies (Krebs et al. 2006; Hlavaty et al. 2008; Munro and Garg 2012). Study duration ranged from 3 to 24 weeks, with 2 of the longer studies being part energy restriction and the remainder weight maintenance diets with continuing supplementation (Krebs et al. 2006; Munro and Garg 2012).

Four of the 6 studies assessing energy restriction plus LCn-3s reported no difference in LBM change between the LCn-3 groups and the control. One study indicated a statistically nonsignificant trend for the LCn-3 group to experience a lower loss of LBM than the control, while total weight loss was similar (Storlien et al. 2001), and 1 study reported a significantly greater loss of LBM for the LCn-3 group when compared with the control; however, group differences at baseline heavily confounded the relatively short trial (Hlavaty et al. 2008).

Thorsdottir et al. (2007) and Abete et al. (2008) assessed the effect of fatty fish intake included in an energy-restricted diet compared with no seafood, while the former also compared lean fish and LCn-3 capsules. Both studies were carried out in otherwise healthy overweight and obese individuals for over 8 weeks. Thorsdottir et al. (2007) reported weight losses of 6.5 and 4.2 kg for the men and women, respectively, for all of the groups, whereas Abete et al. (2008) reported losses of 4.9 kg for both the fatty fish supplemented and the non-fish-supplemented groups; however, there was no reported effect of LCn-3 on LBM change as measured by BIA in either study. Thorsdottir et al. (2007) reported expected changes in RBC fatty acid analysis, whereas Abete et al. (2008) did not report any measure of LCn-3 adherence. The daily dose of total LCn-3s used by Thorsdottir et al. (2007) (1.5 g from capsules and 3 g from fatty fish) was somewhat higher than the relatively small amount used by Abete et al. (2008) (0.15 g EPA,, 0.42 g DHA, 0.62 g docosapentaenoic acid, and 0.5 g α-linolenic acid).

Krebs et al. (2006) (n = 93) and more recently Munro and Garg (2012) (n = 32) assessed the effect of very low energy diets (VLED; ~800 kcal/day) ~835 and 720 kcal/day, followed by prescribed energy balance for 12 and 10 weeks, respectively. Krebs et al. (2006) prescribed 4.2 g of LCn-3s (1.3 g EPA and 2.9 g DHA) for 12 weeks of VLED and found both groups, composed of obese women (21–69 years), lost very little LBM (LCn-3 group, 0.4 kg; and control, 0.5 kg) compared with their total loss of ~10 kg over 12 weeks, determined using DEXA. Considering that the VLED was based primarily on semiskimmed milk, it is possible that the high quality amino-acid intake from the milk may have reduced muscle loss during the trial (Zemel et al. 2005*a*, 2005*b*). Munro and Garg (2012) prescribed 2.04 g of LCn-3 (0.42 g EPA and 1.62 g DHA), yet found no difference in LBM change between groups as measured by BIA.

Storlien et al. (2001) conducted a 3-arm randomised control trial (RCT; n = 52) using individuals with mild hypertension that compared diets of the same total energy (1200 kcal for the females and 1800 kcal for the males) with differing polyunsaturated:saturated fat ratios, i.e., saturated fat (control), 0.25; high LCn-3, 1.0; and high n-6FA, 1.0. Body composition change, as determined by underwater weighing, indicated a nonsignificant tendency for the LCn-3 group to preserve more LBM during weight loss. However, the authors did not report prescribed LCn-3 intake and they did not objectively measure LCn-3 tissue uptake making it difficult to draw conclusions from their findings.

Finally, Hlavaty et al. (2008) performed a 3 week inpatient low energy diet (2500 kJ energy restriction) with or without LCn-3 fortified yoghurt. Despite a total LCn-3 intake of 0.62 g/day and objective LCn-3 markers indicating an increase in a number of tissues, large differences at baseline significantly confounded all results relating to body composition.

Studies of LCn-3s and exercise on change in LBM

To date, 1 study has reported LBM change after a controlled intervention investigating the combined effects of LCn-3s and exercise (Hill et al. 2007). They conducted a high quality 4-arm RCT (n = 65) in middle-aged obese sedentary individuals with at least 1 metabolic comorbidity, such that groups received either LCn-3s or sunflower (placebo) alone or supplementation in conjunction with cardiovascular training of three 45 min treadmill walking/running sessions at 75% VO_{2max} per week for 12 weeks. The LCn-3 groups received 0.36 g EPA and 1.56 g DHA daily. Body composi-

tion was assessed using DEXA, and a significant time interaction existed for fish oil and exercise for body fat mass. LBM change was reported as nonsignificant across all groups.

Summary of LCn-3 supplementation effect on LBM change

From the results of these trials, LCn-3s do not seem to alter LBM when taken alone or in combination with energy restriction or aerobic exercise in generally healthy or obese chronically diseased populations. Limitations in the current literature stem from several key components of the trials completed and relate to dosage of LCn-3s used, length of intervention, assessment of body composition, and the addition of an anabolic stimulus, some of which are discussed below.

Potential limitations of the current interventions and suggestions for the future

Is there a minimum dose required to elicit change in LBM, and how and when should it be measured?

A number of factors are related to tissue concentration of LCn-3s after supplementation. Very little is known regarding optimal LCn-3 concentrations for body composition change, if indeed there is one. In addition, it is not yet known which tissue is most highly correlated to LCn-3 uptake when assessing LBM change. Body mass index (BMI) has been shown to be negatively correlated with tissue concentration increases (Hogg et al. 2006; Yee et al. 2010). Also, studies indicate that higher doses of LCn-3s result in higher maximal tissue concentrations after supplementation (Katan et al. 1997; Yee et al. 2010), while preparation method of the LCn-3s has a significant influence on digestion and absorption of the LCn-3s in the gut.

In regards to BMI, Hogg et al. (2006) reported that while participants in the treatment arm were given the same 4 g daily dose of fish oil (1.88 g EPA and 1.48 g DHA), analysis indicated a 15-fold inter-individual difference in the plasma phospholipid LCn-3:arachadonic acid (AA) ratio from those with the highest and lowest body masses. Both EPA:AA and DHA:AA ratios were significantly correlated with dose per kilogram body mass (for EPA, $r = 0.78$, $p < 0.001$; and for DHA, $r = 0.86$, $p < 0.001$) (Hogg et al. 2006; Yee et al. 2010).

LCn-3 uptake into a variety of tissues has been shown to be dose-dependent. Two long-term studies have reported the effects of increasing doses of LCn-3s taken in the form of triglycerides in men (Katan et al. 1997) and ethyl esters in women (Yee et al. 2010), and both indicated that higher doses resulted in more rapid and higher absolute increases in tissue concentration of LCn-3s. Katan et al. (1997) used LCn-3, delivered as marine-triglycerides, daily doses of 0.97 g (0.81 g EPA and 0.16 g DHA), 1.95 g (1.62 g EPA and 0.33 g DHA), and 2.92 g (2.43 g EPA and 0.49 g DHA) for low, middle, and high groups, respectively, in a group of male monks for 12 months. Tissue content of EPA and DHA were significantly higher for each increment in dose for cholesterol esters, erythrocytes, gluteal adipose, and abdominal adipose tissue at all time points. More recently, Yee et al. (2010) used 4 varying daily doses of 0.84 g (0.47 g EPA and 0.37 g DHA), 2.52 g (1.4 g EPA and 1.12 g DHA), 5.04 g (2.8 g EPA and 2.24 g DHA), and 7.56 g (4.2 g EPA and 3.36 g DHA) over 6 months to determine LCn-3 differences in rate of tissue concentration change of serum total lipids and breast adipose tissue in individuals at high risk of breast cancer. Compared with the 2 lower doses, total serum lipid EPA and DHA concentrations were raised significantly more for the 2 higher doses from 2 to 6 months, while no difference was seen between the 2 higher doses. In contrast, no significant differences were found for the 3 highest doses over 6 months; however, all were significantly higher than the low dose, despite higher doses leading to incrementally higher average values for breast tissue LCn-3s.

Taken together, these long-term dose–response studies indicate LCn-3 concentration in cholesterol esters and erythrocytes increases incrementally with doses of EPA and DHA up to at least 2.95 g/day (2.43 g EPA and 0.49 g DHA) delivered as triglycerides (Katan et al. 1997) and potentially up to 5.04 g/day (2.8 g EPA and 2.24 g DHA) provided as ethyl esters (Yee et al. 2010). While for adipose tissue, maximal tissue content was seen to occur after 1.4 g of EPA and 1.12 g of DHA per day (Yee et al. 2010).

Rate of LCn-3 uptake in different tissues

An additional consideration in LCn-3 supplementation studies is the measurement of LCn-3 uptake into the various body tissues and compartments and how uptake rates can dramatically differ. Typically, tissue content of LCn-3s is measured to determine adherence of consumption, while several epidemiological studies have indicated that LCn-3 tissue concentration may have a prognostic utility for particular conditions. However, different tissues in the body have vastly different uptake rates that need to be considered.

Maximal tissue content of serum cholesterol ester fatty acids has been shown to occur after 56 days of supplementation, with the half-maximum concentration being reached after 4.8 days (Katan et al. 1997). Another compartment commonly measured is plasma phospholipids, which indicate a similar LCn-3 uptake rate; however, for serum erythrocytes, maximum tissue content has been recorded after 60 (Yee et al. 2010) to 180 (Katan et al. 1997) days, with half-maximum levels being reached after 28 to 30 days (Katan et al. 1997; Cao et al. 2006). Several studies consistently indicate that plasma phospholipids (Cao et al. 2006; Harris et al. 2007) and cholesterol esters (Katan et al. 1997) have a higher rate of uptake than erythrocytes (Katan et al. 1997; Cao et al. 2006; Harris et al. 2007), which in turn has a higher rate than that seen in adipose tissue (Katan et al. 1997; Yee et al. 2010). These speeds of uptake logically seem to be related to the rate of turnover for the respective tissues.

Delivery of LCn-3s depending on type of preparation

Limitations of the above studies in dosage and uptake come from the varying uptake of different LCn-3 preparations. Trials have indicated that a hierarchy of LCn-3 uptake exists for different preparations, such that pre-emulsified LCn-3s from fish and normal krill oil are taken up more effectively than LCn-3s as re-esterified triglycerides (marine triglycerides) (Garaiova et al. 2007; Haug et al. 2011; Schuchardt et al. 2011; Ulven et al. 2011), which in turn are better taken up than ethyl esters (Neubronner et al. 2011; Schuchardt et al. 2011; Hussey et al. 2012). These studies are typically conducted over a 48 to 72 h period and good homogeneity in results has been seen. Long-term dose–response studies have not been performed using emulsion or krill oil; however, these forms of delivery are likely to be useful in decreasing the required absolute dose of LCn-3s rather than to improve the effect from LCn-3s when compared with triglyceride and ethyl ester forms (Ulven et al. 2011).

It has not been established what level of LCn-3s or even which tissue will be most advantageous to gauge the likelihood of body composition change. Knowing this may be useful in designing trials that provide definitive results. What can be taken from the existing data is that it takes at least 56 days to saturate the tissue with the highest turnover rate (cholesterol esters) and considerably longer for erythrocytes (180 days) and fat tissue (indefinite). To appropriately determine the effect of LCn-3s a run-in period may be required to maximise tissue concentration before other intervention variables are applied or measured.

Finally, in relation to LBM, the authors could not locate any studies assessing the rate of LCn-3 uptake, e.g., time to half-maximum and (or) maximum saturation for skeletal muscle phospholipids. Some evidence from cancer populations has indicated that LCn-3 muscle phospholipid content is positively correlated with skeletal muscle tissue maintenance during chemotherapy (Murphy et al. 2010); however, LCn-3 dose and time response stud-

ies have not yet been performed in any population. Understanding fatty acid turnover in this compartment is presumably an important consideration for future studies.

How tissue uptake may have affected results from studies assessing change in LBM

In regards to the minimum dosage of omega-3 taken from the current literature cited above, only 3 of the trials assessing the effect of LCn-3s on body composition change provided a dose that would have achieved near maximal tissue uptake of DHA (>1.12 g/day) (Krebs et al. 2006; Hill et al. 2007; Munro and Garg 2012), with only one study providing enough of both EPA (>1.4 g/day) and DHA (Krebs et al. 2006). None of these studies reported a statistically significant difference in LBM change between the groups supplemented with LCn-3 and the control groups. On the other hand, all of these trials were <6 months duration, and whether this timeframe is long enough for LCn-3s to have an effect on LBM is currently unknown. Some data indicate that red blood cell (RBC) content of LCn-3 relates to changes in fat mass (Munro and Garg 2012), but equivalent data are not yet available for LBM, and so this is an area that requires further investigation.

Does the addition of LCn-3s to an anabolic stimulus (e.g., an amino acid dose or exercise program) have a synergistic effect on LBM or LBM function?

Traditionally, weight, BMI, and measures of body fat (percent and waist circumference) have been positively correlated with general incidence of disease and mortality. However, over the past decade, significantly more attention has been paid to the importance of LBM and the function of that LBM to determine even stronger relationships with disease and mortality in certain populations (Newman et al. 2005, 2006; Taaffe 2006; Haran et al. 2012). For this reason, measurement of not only muscle mass, but muscle function may have significant clinical utility both on an individual and population health level.

LBM function rather than absolute LBM as a prognostic health marker

The Analysis of the Health, Ageing, and Body Composition trial by Newman et al. (2006) indicated that muscle strength as determined by quadriceps strength and hand-grip strength were both independently correlated to mortality risk, and remained so when analyses were adjusted for sarcopenia. In addition, the strength–mortality relationship remained statistically significant after controlling for levels of inactivity, chronic disease, and race (Newman et al. 2006). This study corroborated and added strength to previous findings as they used cross-sectional area of the thigh muscle (CT scan) and appendicular muscle mass (DEXA) measures to control for sarcopenia. Similar findings were reported in a male cohort (n = 8762) followed for 18.9 years: when compared with the lowest tertile, a statistically significant reduction in all-cause and cancer-mortality was seen for those in the middle and highest tertile of upper and lower body combined strength. These remained significant after adjusting for relevant confounders including cardiorespiratory fitness as measured by VO_{2max} (Ruiz et al. 2008). Furthermore, the accumulation of intramuscular fat, in a non-elite athlete setting, has been associated with insulin resistance and increased cardiovascular disease risk (Goodpaster et al. 2000). Taken together, function and quality of the muscle is building as an important factor in general survival and quality of life.

Is there synergy between LCn-3s and an anabolic stimulus?

Although LCn-3s may not have a convincing role in promoting LBM gains in the trials published to date, recent data have indicated a role for LCn-3s in enhanced muscle quality (Murphy et al. 2011*a*; 2011*b*), response to resistance training, and muscle protein synthesis in both young and healthy and older populations when an anabolic stimulus, essential amino acid/insulin (Smith et al. 2011*a*, 2011*b*) or resistance exercise (Rodacki et al. 2012) is added to LCn-3 supplementation (Table 2).

Smith et al. (2011*a*, 2011*b*) performed 2 small high-quality studies in young + healthy (n = 9, 1-arm pre/post-test), and older + healthy (n = 16, 2-arm RCT) men and women to assess the effect of 8 weeks LCn-3 (1.86 g EPA/day and 1.5 g DHA/day (for both studies)) or corn oil supplementation on muscle protein synthesis during basal postabsorptive and hyperinsulineamic–hyperaminoacidaemic clamp conditions. Interestingly, for both groups, LCn-3 supplementation did not increase muscle protein synthesis in the basal postabsorptive state. However, the rates of muscle protein synthesis in response to insulin and amino acid infusion were ~50% and 100% increases in young and older populations, respectively. Of note, inflammatory markers of both populations were unaffected through the 8 weeks, while an upregulation of anabolic signalers mTOR and p70s6k found in the LCn-3 groups only provided a possible mechanism by which LCn-3s may have an anabolic effect. The findings were that while LCn-3s alone did not seem to increase basal protein synthesis, in conjunction with other anabolic stimuli, i.e., amino acids and insulin, the anabolic response was enhanced. Both of these studies contribute to the hypothesis that LCn-3s may act as a muscle tissue anabolic primer, such that an anabolic stimulus elicits a greater response than when there is less LCn-3s in the muscle tissue.

Rodacki et al. (2012) investigated the neuromuscular effects of LCn-3 supplementation in ~65-year-old women (n = 45) and how length of supplementation affected different elements of muscle contraction. The study involved 3 groups, all received 36 sessions of progressive lower body strength training over 12 weeks. Group 1 received resistance training alone, group 2 had resistance training and daily LCn-3 supplementation (0.36 g EPA and 0.24 g DHA) for 12 weeks, while the group 3 received the LCn-3 dose for 60 days leading into the study and then continued on the same dose for the 12 weeks with resistance training. All groups experienced improvements in peak torque, electromechanical delay, and functional capacity. Interestingly, both of the groups supplemented with LCn-3 experienced significantly greater improvements in peak torque, electromechanical delay, and in 1 of 4 functional tests. However, no differences were found among the LCn-3 supplemented groups themselves. While the population and dosage were small, confounding issues due to diet and physical activity were addressed, and the trial was of appropriate length to realise benefit.

This trial generated 2 important points for further investigation. Firstly, small doses of LCn-3s had a positive effect on neural tissue when combined with exercise. Secondly, LCn-3s alone did not seem to change the neurological response of the participants who had 60 days of lead-in supplementation. No differences were seen at baseline for any variable; only when resistance training as an additional anabolic stimulus was applied did the neural tissue have greater responsiveness when compared with the control groups. Considering that it is typically the higher force generating type II fibres that are negatively affected in myopenia (Taaffe 2006), improving muscle protein synthetic and neurologic responses to training have useful implications in older and deconditioned populations. Thus, future research determining the LCn-3 benefits when in combination with progressive resistance exercise is warranted.

Finally, Murphy et al. (2011*a*, 2011*b*) recently investigated the effects of LCn-3s on muscle quality in cancer survivor populations undergoing chemotherapy. This group used CT images as part of routine therapy to detail very accurate estimations of LBM, and in addition, these images allow quantitative assessment of triglyceride infiltration of the muscle tissue.

Initial observations in patients undergoing 10 weeks of chemotherapy for nonsmall cell lung cancer showed that patients classified as sarcopenic (low skeletal muscle mass values as assessed

Table 2. Studies assessing the effect of LCn-3s on LBM function and quality.

Population, study design, and sample size	Dose of LCn-3 and duration	Anabolic stimulus	Measure of LBM/response	Result
Healthy young (25–45 years), single arm pre–post study, $n = 9$ (Smith et al. 2011*a*, 2011*b*)	EPA, 1.86 g; DHA, 1.5 g; 8 weeks	Hyperinsulinaemic–hyperaminoacidaemic clamp	MPS (biopsy), stable isotope tracer techniques	~50% ↑ in mTOR and p70s6k at 8 weeks compared with baseline. No change in basal post-absorptive MPS after 8 weeks
Older healthy (71.5 years), 2-arm RCT, $n = 16$ (Smith et al. 2011*a*, 2011*b*)	EPA, 1.86 g; DHA, 1.5 g; control, corn oil; 8 weeks	Hyperinsulinaemic–hyperaminoacidaemic clamp	MPS (biopsy), stable isotope tracer techniques	~100% ↑ in MPS in the LCn-3 supplemented group after clamp only. No change in basal post-absorptive MPS
Healthy women (64 years), 3-arm RCT, $n = 45$ (Rodacki et al. 2012)	EPA, 0.32 g; DHA, 0.24 g; 12 weeks; group 1, control; group 2: 12 weeks of LCn-3 supplementation; group-3, 60 days LCn-3 supplementation before week 0 + 12 weeks of LCn-3 supplementation	Twelve weeks lower body progressive resistance training (3 sessions/week)	Peak torque, EMD, and functional capacity	Statistically significant improvements in peak torque and EMD and 1 of 4 functional tests for both LCn-3 supplemented groups vs. the control. No difference among the LCn-3 supplemented groups themselves
NSCLC (~64 years), 2-arm self-selected group, $n = 40$ (Murphy et al. 2011, 2011*b*)	EPA, 2.2 g; control, standard care; 10 weeks	Catabolic stimulus (chemotherapy)	CT scan (2 consecutive slices at L3), IMTG calculated	Gain in EPA content correlated to LBM gain ($r^2 = 0.55$, $p = 0.01$). IMTG ↑ in standard care (+9.5% over 100 days vs. –16.4% over 100 days)

Note: LCn-3s, long-chain omega-3 fatty acids; LBM, lean body mass; EPA, eicosapentanoic acid; DHA, docosapentanoic acid; MPS, muscle protein synthetic; RCT, randomised control trial; EMD, electromechanical delay; NSCLC, nonsmall cell lung cancer; IMTG, intramyocellular triglycerides.

by CT scan; men, <55.4 cm^2/m^2 and women, <39.9 cm^2/m^2), or those who experienced the greatest muscle loss (>–10% over 100 days) during treatment, had significantly lower content of EPA, DHA, and total LCn-3s in their plasma phospholipids (Murphy et al. 2010). Following this, in a similar population, supplementation of 2.2 g EPA (choice of capsules or liquid was given) or standard care (no EPA) were compared over 10 weeks of treatment. When compared with standard care, those consuming EPA experienced significantly better mass retention (–2.3 ± 2.6 kg vs. +0.5 ± 1 kg, $p < 0.05$, respectively) and muscle mass rate of change (–6.8% ± 2.6% per 100 days vs. +0.1% ± 1.6% per 100 days, respectively). Of note, the rate of change of intramuscular triglycerides was significantly more favourable in the group supplemented with LCn-3 when compared with standard care (–16.4% ± 13.9% per 100 days vs. +9.5% ± 5.2% per 100 days, respectively). Taken together, LCn-3s were positively associated with muscle quality over the treatment period, as measured by intramyocellular triglycerides. While no specific measures of muscle strength were assessed, a partially overlapping population studied by the same group indicated LCn-3 supplementation was correlated with a 2-fold improvement in treatment response and improved treatment completion rates (Murphy et al. 2011*a*, 2011*b*).

Discussion

The current literature does not support a clinically meaningful effect from LCn-3s alone for change in LBM alone or in conjunction with energy restriction using doses up to 1.3 g EPA/day and 2.9 g DHA/day over 6 months. In addition, a high dose of DHA LCn-3 (1.56 g/day) seems to have no effect on LBM in conjunction with aerobic training after 12 weeks.

The minimum dose of LCn-3s required to elicit change in LBM is currently unknown; however, available evidence indicates that trials may need to use at least 1.4 g EPA and 1.12 g DHA for at least 1 month to establish a high tissue saturation of LCn-3s. Until these studies are conducted, definitive conclusions cannot be made.

Higher daily doses result in higher and more rapid tissue saturation; however, it is not currently known which tissue or compartment is most relevant to LBM change. Studies investigating anabolic response to exercise after LCn-3 supplementation did not correlate muscle phospholipid content of LCn-3 to changes in muscle physiology. In addition, neither dose–response nor duration have been assessed for muscle phospholipid content. However, a significant change in LBM physiology was seen after 8 weeks of LCn-3 supplementation.

In healthy young and older populations, the current evidence indicates that LCn-3s may enhance muscle protein synthesis and neural responses when combined with an anabolic stimulus, hyperaminoacidaemic–hyperinsulinaemic clamp, and resistance training, respectively. Furthermore, preliminary evidence indicates that LCn-3s alone do not seem to improve LBM function in healthy populations, rather they may have a more permissive role, allowing muscle tissue to better respond to an anabolic stimulus. In contrast, in typically catabolic populations, i.e., individuals undergoing chemotherapy, LCn-3s may reduce LBM loss and intramyocellular triglyceride infiltration, while this may improve chemotherapy response in nonsmall cell lung cancer, it is not known whether these improvements are related to better physical function.

It is recognised that Western diets have considerably lower LCn-6:LCn-3 ratios than is considered optimal (15–20:1 compared with 1–4:1) (Calder 2009). The data reviewed above indicates a change in muscle function or quality after supplementation; however, it is unknown whether the initial and poorer state of the muscle was a result of LCn-3 deficiency, and that supplementation acted only to restore optimal concentration, as opposed to the suggestion that LCn-3s have a truly therapeutic effect comparable with that of drugs. In populations experiencing significant catabolism, significant doses of LCn-3s have been shown to have a therapeutic ef-

fect. However, in healthier populations, a relatively low dose of LCn-3s has elicited change in LBM function. Thus, LCn-3's true therapeutic value may be reserved for populations with significant myopenia, while in contrast, healthy populations may be assisted by establishing optimal LCn-3 levels. While future studies should aim to tease these differences out, supplementation with LCn-3s is safe, and is an important consideration in a number of populations.

References

Abete, I., Parra, D., Crujeiras, A.B., Goyenechea, E., and Martinez, J.A. 2008. Specific insulin sensitivity and leptin responses to a nutritional treatment of obesity via a combination of energy restriction and fatty fish intake. J. Hum. Nutr. Diet. **21**(6): 591–600. doi:10.1111/j.1365-277X.2008.00902.x. PMID:18759956.

Academy of Nutrition and Dietetics. 2010. Academy of Nutrition and Dietetics evidence analysis manual. (Available from andevidencelibrary.com/topic.cfm?cat=1155&auth=1).

Babcock, T.A., Kurland, A., Helton, W.S., Rahman, A., Anwar, K.N., and Espat, N.J. 2003. Inhibition of activator protein-1 transcription factor activation by omega-3 fatty acid modulation of mitogen-activated protein kinase signaling kinases. J. Parenter. Enteral Nutr. **27**(3): 176–180. doi:10.1177/0148607103027003176. PMID:12757110.

Breen, L., and Phillips, S.M. 2011. Skeletal muscle protein metabolism in the elderly: Interventions to counteract the 'anabolic resistance' of ageing. Nutr. Metab. **8**(1): 68–68. doi:10.1186/1743-7075-8-68. PMID:21975196.

Buckley, J.D., and Howe, P.R.C. 2009. Anti-obesity effects of long-chain omega-3 polyunsaturated fatty acids. Obes. Rev. **10**(6): 648–659. doi:10.1111/j.1467-789X.2009.00584.x. PMID:19460115.

Buckley, J.D., and Howe, P.R.C. 2010. Long-chain omega-3 polyunsaturated fatty acids may be beneficial for reducing obesity — a review. Nutrients, **2**(12): 1212–1230. doi:10.3390/nu2121212. PMID:22254005.

Bunout, D., de la, Maza, M.P., Barrera, G., Leiva, L., and Hirsch, S. 2011. Association between sarcopenia and mortality in healthy older people. Australas J. Ageing, **30**(2): 89–92. doi:10.1111/j.1741-6612.2010.00448.x. PMID:21672118.

Calder, P.C. 2009. Polyunsaturated fatty acids and inflammatory processes: New twists in an old tale. Biochimie, **91**(6): 791–795. doi:10.1016/j.biochi.2009.01.008. PMID:19455748.

Cao, J., Schwichtenberg, K.A., Hanson, N.Q., and Tsai, M.Y. 2006. Incorporation and clearance of omega-3 fatty acids in erythrocyte membranes and plasma phospholipids. Clin. Chem. **52**(12): 2265–2272. doi:10.1373/clinchem.2006.072322. PMID:17053155.

Castaneda, C., Charnley, J.M., Evans, W.J., and Crim, M.C. 1995. Elderly women accommodate to a low-protein diet with losses of body cell mass, muscle function, and immune response. Am. J. Clin. Nutr. **62**(1): 30. PMID:7598064.

Cerchietti, L.C.A., Naviganteac, A.H., and Castroa, M.A. 2007. Effects of eicosapentaenoic and docosahexaenoic n-3 fatty acids from fish oil and preferential cox-2 inhibition on systemic syndromes in patients with advanced lung cancer. Nutr. Cancer, **59**(1): 14–20. doi:10.1080/01635580701365068. PMID:17927497.

Chapkin, R.S., Kim, W., Lupton, J.R., and McMurray, D.N. 2009. Dietary docosahexaenoic and eicosapentaenoic acid: Emerging mediators of inflammation. Prostaglandins Leukot. Essent. Fatty Acids, **81**: 187–191. doi:10.1080/01635580701365068. PMID:19502020.

Couet, C., Delarue, J., Ritz, P., Antoine, J.-M., and Lamisse, F. 1997. Effect of dietary fish oil on body fat mass and basal fat oxidation in healthy adults. Int. J. Obes. **21**: 637–643. doi:10.1038/sj.ijo.0800451. PMID:15481762.

Crochemore, I.C.C., Souza, A.F.P., de, Souza, A.C.F., and Rosado, E.L. 2012. ω-3 Polyunsaturated fatty acid supplementation does not influence body composition, insulin resistance, and lipemia in women with type 2 diabetes and obesity. Nutr. Clin. Pract. **27**(4): 553–560 doi:10.1177/0884533612444535. PMID:22661243.

Deutz, N.E.P., Safar, A., Schutzler, S., Memelink, R., Ferrando, A., Spencer, H., et al. 2011. Muscle protein synthesis in cancer patients can be stimulated with a specially formulated medical food. Clin. Nutr. **30**(6): 759–768. doi:10.1016/j.clnu.2011.05.008. PMID:21683485.

Edwards, I.J., and O'Flaherty, J.T. 2008. Omega-3 fatty acids and PPARγ in cancer. PPAR Res. 2008 Article ID: 358052 doi: doi:10.1155/2008/358052. PMID: 18769551.

Fearon, K.C.H., Evans, W.J., and Anker, S.D. 2011*a*. Myopenia — a new universal term for muscle wasting. J. Cachexia Sarcopenia Muscle, **2011**(2): 1–3. doi:10.1007/s13539-011-0025-7. PMID:21475620.

Fearon, K., Strasser, F., Anker, S.D., Bosaeus, I., Bruera, E., Fainsinger, R.L., et al. 2011b. Definition and classification of cancer cachexia: an international consensus. Lancet Oncol. **12**(5): 489–495. doi:10.1016/S1470-2045(10)70218-7. PMID:21296615.

Garaiova, I., Guschina, I.A., Plummer, S.F., Tang, J., Wang, D., and Plummer, N.T. 2007. A randomised cross-over trial in healthy adults indicating improved absorption of omega-3 fatty acids by pre-emulsification. Nutr. J. **6**(4). doi:10.1186/1475-2891-6-4. PMID:17254329.

Genton, L., Graf, C.E., Karsegard, V.L., Kyle, U.G., and Pichard, C. 2012. Low fat-free mass as a marker of mortality in community-dwelling healthy elderly subjects. Age Ageing. doi:10.1093/ageing/afs091. PMID:22789764.

Goodpaster, B.H., Thaete, F.L., and Kelley, D.E. 2000. Thigh adipose tissue distribution is associated with insulin resistance in obesity and in type 2 diabetes mellitus. Am. J. Clin. Nutr. **71**(4): 885–892. PMID:10731493.

Hamdy, O., Porramatikul, S., and Al-Ozairi, E. 2006. Metabolic obesity: The paradox between visceral and subcutaneous fat. Curr. Diabetes Rev. **2**(4): 367–373. doi:10.2174/1573399810602040367. PMID:18220642.

Haran, P., Rivas, D., and Fielding, R. 2012. Role and potential mechanisms of anabolic resistance in sarcopenia. J. Cachexia Sarcopenia Muscle. 1–6. doi:10.1007/s13539-012-0068-4. PMID:22589021.

Harris, W.S., Pottala, J.V., Sands, S.A., and Jones, P.G. 2007. Comparison of the effects of fish and fish-oil capsules on the n-3 fatty acid content of blood cells and plasma phospholipids. Am. J. Clin. Nutr. **86**(6): 1621–1625. PMID:18065578.

Haug, I.J., Sagmo, L.B., Zeiss, D., Olsen, I.C., Draget, K.I., and Seternes, T. 2011. Bioavailability of EPA and DHA delivered by gelled emulsions and soft gel capsules. Eur. J. Lipid Sci. Technol. **113**(2): 137–145. doi:10.1002/ejlt.201000450.

Hill, A., Buckley, J.D., Murphy, K.J., and Howe, P.R.C. 2007. Combining fish-oil supplements with regular aerobic exercise improves body composition and cardiovascular disease risk factors. Am. J. Clin. Nutr. **85**: 1267–1274. PMID:17490962.

Hlavaty, P., Kunesova, M., Gojova, M., Tvrzicka, E., Vecka, M., Roubal, P., et al. 2008. Change in fatty acid composition of serum lipids in obese females after short-term weight-reducing regimen with the addition of n-3 long chain polyunsaturated fatty acids in comparison to controls. Physiol. Res. **57**(Suppl. 1): S57–S65. PMID:18271691.

Hogg, R.J., Fitzgibbons, L., Atkins, C., Nardelli, N., and Bay, R.C. (North American IqA Nephropathy Study Group). 2006. Efficacy of omega-3 fatty acids in children and adults with iga nephropathy is dosage and size-dependent. Clin. J. Am. Soc. Nephrol. **1**: 1167–1172. doi:10.2215/CJN.02300606. PMID:17699343.

HunCha, S., Fukushima, A., Sakuma, K., and Kagawa, Y. 2001. Chronic docosahexaenoic acid intake enhances expression of the gene for uncoupling protein 3 and affects pleiotropic mrna levels in skeletal muscle of aged c57bl/6njcl mice. J. Nutr. **131**(1): 2636–2642. PMID:11584083.

Hussey, E.K., Portelli, S., Fossler, M.J., Gao, F., Harris, W.S., Blum, R.A., et al. 2012. Relative bioavailability of an emulsion formulation for omega-3-acid ethyl esters compared to the commercially available formulation. Clin. Pharmacol. Drug Devel. **1**(1): 14–23. doi:10.1177/2160763X11431107.

Katan, M.B., Deslypere, J.P., vanBirgelen, A.P., Penders, M., and Zegwaard, M. 1997. Kinetics of the incorporation of dietary fatty acids into serum cholesteryl esters, erythrocyte membranes, and adipose tissue: an 18-month controlled study. J. Lipid Res. **38**(10): 2012–2022. PMID:9374124.

Krebs, J.D., Browning, L.M., McLean, N.K., Rothwell, J.L., Mishra, G.D., Moore, C.S., and Jebb, S.A. 2006. Additive benefits of long-chain n-3 polyunsaturated fatty acids and weight-loss in the management of cardiovascular disease risk in overweight hyperinsulinaemic women. Int. J. Obes. **30**(10): 1535–1544. doi:10.1038/sj.ijo.0803309. PMID:16552404.

Lenk, K., Schuler, G., and Adams, V. 2010. Skeletal muscle wasting in cachexia and sarcopenia: molecular pathophysiology and impact of exercise training. J. Cachexia Sarcopenia Muscle, **1**(1): 9–21. doi:10.1007/s13539-010-0007-1. PMID:21475693.

Martínez-Victoria, E., and Yago, M.D. 2012. Omega 3 polyunsaturated fatty acids and body weight. Br. J. Nutr. **107**(Suppl. 2): S107. doi:10.1017/S000711451200150x. PMID:22591885.

Mishra, A., Chaudhary, S., and Sethi, S. 2004. Oxidized omega-3 fatty acids inhibit NF-κB activation via a PPARα-dependent pathway. Arterioscler. Thromb. Vasc. Biol. **24**(9): 1621–1627. doi:10.1161/01.ATV.0000137191.02577.86. PMID:15231516.

Munro, I.E., and Garg, M.L. 2012. Dietary supplementation with n-3 PUFA does not promote weight loss when combined with a very-low-energy diet. Br. J. Nutr. **3**: 1–9. doi:10.1017/S0007114511006817. PMID:15392404.

Murphy, R.A., Mourtzakis, M., Chu, Q.S., Reiman, T., and Mazurak, V.C. 2010. Skeletal muscle depletion is associated with reduced plasma (n-3) fatty acids in non-small cell lung cancer patients. J. Nutr. **140**(9): 1602–1606. doi:10.3945/jn.110.12352. PMID:20631325.

Murphy, R.A., Mourtzakis, M., Chu, Q.S.C., Baracos, V.E., Reiman, T., and Mazurak, V.C. 2011. Nutritional intervention with fish oil provides a benefit over standard of care for weight and skeletal muscle mass in patients with nonsmall cell lung cancer receiving chemotherapy. Cancer, **117**(8): 1775–1782. doi:10.1002/cncr.25709. PMID:21360698.

Murphy, R.A., Mourtzakis, M., Chu, Q.S.C., Baracos, V.E., Reiman, T., and Mazurak, V.C. 2011. Supplementation with fish oil increases first-line chemotherapy efficacy in patients with advanced nonsmall cell lung cancer. Cancer, **117**(16): 3774–3780. doi:10.1002/cncr.25933. PMID:21328326.

Muscaritoli, M., Anker, S.D., Argilés, J., Aversa, Z., Bauer, J.M., Biolo, G., et al. 2010. Consensus definition of sarcopenia, cachexia and pre-cachexia: Joint document elaborated by Special Interest Groups (SIG) "cachexia-anorexia in chronic wasting diseases" and "nutrition in geriatrics". Clin. Nutr. **29**(2): 154–159. doi:10.1016/j.clnu.2009.12.004. PMID:20060626.

Neubronner, J., Schuchardt, J.P., Kressel, G., Merkel, M., von, Schacky, C., and Hahn, A. 2011. Enhanced increase of omega-3 index in response to long-term

n-3 fatty acid supplementation from triacylglycerides versus ethyl esters. Eur. J. Clin. Nutr. **65**(2): 247–254. doi:10.1038/ejcn.2010.239. PMID:21063431.

Newman, A.B., Sun Lee, J., Visser, M., J.S., Visser, M., Goodpaster, B.H., Kritchevsky, S.B., Tylavsky, F.A., et al. 2005. Weight change and the conservation of lean mass in old age: the Health, Aging and Body Composition Study. Am. J. Clin. Nutr. **82**(4): 872–878. PMID:16210719.

Newman, A.B., Kupelian, V., Visser, M., Simonsick, E.M., Goodpaster, B.H., Kritchevsky, S.B., et al. 2006. Strength, but not muscle mass, is associated with mortality in the Health, Aging and Body Composition Study Cohort. J. Gerontol. A Biol. Sci. Med. Sci. **61**(1): 72–77. doi:10.1093/gerona/61.1.72. PMID:16456196.

Noreen, E.E., Sass, M.J., Crowe, L., Pabon, V.A., Brandauer, J., and Averil, L.K. 2010. Effects of supplemental fish oil on resting metabolic rate, body composition, and salivary cortisol in healthy adults. J. Int. Soc. Sports Nutr. **7**(31). doi:10.1186/1550-2783-7-31. PMID:20932294.

Novak, T.E., Babcock, T.A., Jho, D.H., Helton, W.S., and Espat, N.J. 2003. NF-κB inhibition by ω-3 fatty acids modulates LPS-stimulated macrophage TNF-α transcription. Am. J. Physiol. Lung Cell Mol. Physiol. **284**(1): L84–L89. PMID:12388359.

Rodacki, C.L., Rodacki, A.L., Pereira, G., Naliwaiko, K., Coelho, I., Pequito, D., and Fernandes, L.C. 2012. Fish-oil supplementation enhances the effects of strength training in elderly women. Am. J. Clin. Nutr. **95**(2): 428–436. doi:10.3945/ajcn.111.02191. PMID:22218156.

Rogers, E.S., MacLeod, R.D., Stewart, J., Bird, S.P., and Keogh, J.W.L. 2011. A randomised feasibility study of EPA and Cox-2 inhibitor (Celebrex) versus EPA, Cox-2 inhibitor (Celebrex), resistance training followed by ingestion of essential amino acids high in leucine in NSCLC cachectic patients — ACCeRT study. BMC Cancer, **11**(1): 493–493. doi:10.1186/1471-2407-11-493. PMID:22111896.

Roubenoff, R. 2008. Molecular basis of inflammation: relationships between catabolic cytokines, hormones, energy balance, and muscle. J. Parenter. Enteral Nutr. **32**(6): 630–632. doi:10.1177/0148607108324875. PMID:18974242.

Ruiz, J.r., Sui, X., Lobelo, F, Morrow, J., Jr., Jackson, A., Sjöström, M., and Blair, S.N. 2008. Association between muscular strength and mortality in men: prospective cohort study. Br. Med. J. **337**(7661): a439–a495. doi:10.1136/bmj.a439. PMID:18595904.

Schuchardt, J., Schneider, I., Meyer, H., Neubronner, J., von Schacky, C., and Hanh, A. 2011. Incorporation of EPA and DHA into plasma phospholipids in response to different omega-3 fatty acid formulations — a comparative bioavailability study of fish oil vs. krill oil. Lipids Health Dis. **10**(1): 145. doi:10.1186/1476-511X-10-145. PMID:21854650.

Simopoulos, A.P. 1999. Evolutionary aspects of omega-3 fatty acids in the food supply. Prostaglandins Leukot. Essent. Fatty Acids, **60**(5): 421–429. doi:10.1016/S0952-3278(99)80023-4. PMID:10471132.

Singer, S.J. 2004. Some early history of membrane molecular biology. Annu. Rev. Physiol. **66**: 1–27. doi:10.1146/annurev.physiol.66.032902.131835. PMID:14977394.

Smith, G.I., Atherton, P., Reeds, D.N., Mohammed, B.S., Rankin, D., Rennie, M.J., and Mittendorfer, B. 2011. Dietary omega-3 fatty acid supplementation increases the rate of muscle protein synthesis in older adults: a randomized controlled trial. Am. J. Clin. Nutr. **93**: 402–412. doi:10.3945/ajcn.110.005611. PMID:21159787.

Smith, G.I., Atherton, P., Reeds, D.N., Mohammed, B.S., Rankin, D., Rennie, M.J., and Mittendorfer, B. 2011. Omega-3 polyunsaturated fatty acids augment the muscle protein anabolic response to hyperinsulinaemia–hyperaminoacidaemia in healthy young and middle-aged men and women. Clin. Sci. **121**(6): 267–278. doi:10.1042/CS20100597. PMID:21501117.

Stenholm, S., Harris, T.B., Rantanen, T., Visser, M., Kritchevsky, S.B., and Ferruci, L. 2008. Sarcopenic obesity: definition, etiology and consequences. Curr. Opin. Clin. Nutr. Metab. Care, **11**(6): 693–700. doi: 10.1097/MCO.0b013e328312c37d. PMID:18827572.

Storlien, L.H., Robertson, R.M., Hill, J.O., Kriketos, A.D., Robertson, R.M., Sharp, T.A., Drougas, H., Reed, G.W., Storlien, L.H., and Hill, J.O. 2001. Role of weight loss and polyunsaturated fatty acids in improving metabolic fitness in moderately obese, moderately hypertensive subjects. J. Hypertens. **19**(10): 1745–1754. doi:10.1097/00004872-200110000-00007. PMID:11593093.

Taaffe, D.R. 2006. Sarcopenia: exercise as a treatment strategy. Aust. Fam. Physician, **35**(3): 130–134. PMID:16525526.

Thorsdottir, I., Tomasson, H., Gunnarsdottir, I., Gisladottir, E., Kiely, M., Parra, M.D., et al. 2007. Randomized trial of weight-loss-diets for young adults varying in fish and fish oil content. Int. J. Obes. **31**(10): 1560–1566. doi:10.1038/sj.ijo.0803643. PMID:17502874.

Ulven, S., Kirkhus, B., Lamglait, A., Basu, S., Ellind, E., Haider, T., et al. 2011. Metabolic effects of krill oil are essentially similar to those of fish oil but at lower dose of EPA and DHA, in healthy volunteers. Lipids, **46**(1): 37–46. doi:10.1007/s11745-010-3490-4. PMID:21042875.

Wang, C., Harris, W.S., Chung, M., Lichtenstein, A.H., Balk, E.M., Kupelnick, B., et al. 2006. n-3 Fatty acids from fish or fish-oil supplements, but not α-linolenic acid, benefit cardiovascular disease outcomes in primary- and secondary-prevention studies: a systematic review. Am. J. Clin. Nutr. **84**(1): 5–17. PMID:16825676.

Yee, L.D., Lester, J.L., Cole, R.M., Richardson, J.R., Hsu, J.C., Li, Y., et al. 2010. x-3 Fatty acid supplements in women at high risk of breast cancer have dose-dependent effects on breast adipose tissue fatty acid composition. Am. J. Clin. Nutr. **91**: 1185–1194. doi:10.3945/ajcn.2009.29036. PMID:20335550.

Zemel, M.B., Richards, J., Mathis, S., Milstead, A., Gebhardt, L., and Silva, E. 2005. Dairy augmentation of total and central fat loss in obese subjects. Int. J. Obes. Relat. Metab. Disord. **29**(4): 391–397. doi:10.1038/sj.ijo.0802880. PMID:15672113.

Zemel, M.B., Richards, J., Milstead, A., and Campbell, P. 2005. Effects of calcium and dairy on body composition and weight loss in African-American Adults. Obes. Res. **12**: 1218–1225. PMID:16076991.

2.5.1 Summary of findings from Published Manuscript #2

This review compiled the latest studies describing the effects of LCn-3 on LBM. From the literature, if LCn-3s to have an effect on LBM in non-cancer populations, it seems they need to be taken in conjunction with an anabolic stimulus. LCn-3 supplementation had no effect on LBM when combined with diet alone. However when combined with an acute anabolic stimulation through hormonal change or physical work (fed state amino acids and insulin or resistance training, respectively) changes in LBM and LBM function were observed. Indeed, in terms of LBM, LCn-3s are seen to be permissive and synergistic of change in LBM and LBM function, as opposed to being a primary driver for the change. Of note, in the case of the experiments performed by Smith et al (2011), the change in muscle protein synthesis seen in the LCn-3 group was not related to change in inflammation, thus additional pathways need to be investigated.

Findings from this manuscript emphasise the need for further research into the effects of combining exercise and diet therapies. Some benefit may be found, however, these studies indicate the most efficient improvement in LBM will be found when the synergies of these modalities are harnessed appropriately.

Finally, the review of dosing strategies indicates that when using a tissue level of LCn-3 to measure adherence, the type of tissue, dose and length of supplementation time is critical. Erythrocyte fatty acid content presents as an excellent marker for short to mid-term supplementation and uptake. A dose suitable to maximise uptake into this tissue is very important for studies of eight to 24 weeks in length.

2.6 Overview of literature review and rationale of study

Breast cancer survivors experience adverse body composition changes following treatment. Absolute body weight gains of 2-5kg are still common after treatment, however this has improved in the last decade due to greater awareness and improved treatment. Weight gains often occur in conjunction with a concurrent loss of LBM and deposition of both visceral and subcutaneous fat mass. The changes are most dramatic in premenopausal and perimenopausal breast cancer survivors, however postmenopausal women experience similar changes, albeit at a lower magnitude. In addition, the highest rate of body composition change occurs during or soon (6-12months) after treatment has finished. Similar body composition changes in non-cancer populations are associated with increased risk of cardiovascular and metabolic syndrome related diseases. Cardio-metabolic disease related mortality is now higher than breast cancer-related mortality for those with a history of breast cancer, thus attention to the known risk factors of this group of diseases is a priority for this population.

Currently, the understanding of the mechanisms underpinning LBM change is limited by a lack of research. At this stage, taking AIs may have a positive effect on LBM, and it may act synergistically with exercise. LBM loss may be a result of chronic inflammation that may be partly induced by chemotherapy related myotoxicity, and/or physical inactivity. However, neither of these theories have been rigorously investigated in a breast cancer population. Given the benefits of physical activity to LBM maintenance, measuring muscle function variables as markers of physical activity may provide insights into modifiable risk factors associated with LBM loss.

Interventions evaluating the effects of exercise during or following breast cancer treatment have shown that LBM loss may be halted and reversed after exercise training. Dietary interventions, while successful in reducing body weight have been shown to lead to increasing incidence of myopenia and LBM loss. For a population already at risk of decreased muscle mass, further losses that are typical of dietary energy restriction may be contraindicated. However, regardless of changes in LBM, total weight loss was shown to reduce classical risk factors of cardiovascular disease, such as blood lipids, triglycerides and waist girth.

Combining aerobic and resistance exercise have been shown to improve body weight, body fat% and waist girth loss with maintenance of LBM. However, these interventions have been limited by poor description of the exercise protocols.

An area that has not been investigated in breast cancer populations is the addition of nutrients known to have anabolic synergies with exercise or dietary interventions. LCn-3s are linked to a decrease in muscle wasting in advanced cancer patients, and recent evidence indicates they may have synergistic effects with other anabolic agents such as resistance training, and elevated amino

acid/insulin concentrations. Furthermore, LCn-3s alone and in conjunction with exercise have been shown to reduce fat mass and may do this while preventing LBM in non-cancer populations. Of note, benefits for LBM seen in advanced cancer patients seemed to be linked to adequate EPA content in the plasma, while improvements in body fat% in non-cancer populations may be primarily derived from DHA LCn-3. Considering breast cancer survivors share medical interventions with cancer populations and metabolic outcomes of those with cardio-metabolic conditions, an adequate dose of 1.4g of EPA and 1.12g of DHA per day, may be effective in preventing adverse body composition changes in breast cancer survivors particularly when combined with structured exercise.

With consideration to the current literature, a theoretical model (Figure 2.1) was developed to outline factors that influence body composition after a breast cancer diagnosis. The model includes established treatment and intermediary factors, and established and hypothesised interventions related to body composition change after a diagnosis for breast cancer.

Currently, there is limited knowledge regarding the associations of objective measures of physical activity or erythrocyte fatty acids and how they could predict LBM after completion of breast cancer treatment.

In addition, no studies have assessed the influence of LCn-3 and resistance training on LBM changes in any population. Recognising this gap, and understanding the potential benefits that LCn-3s may confer for this large population of women, this study aims:

1. To investigate the effectiveness of LCn-3 supplementation alone compared to a nutrition and exercise lifestyle program, and to determine if there is further benefit for LBM when LCn-3s are combined with the nutrition and exercise lifestyle program.
2. To investigate the lifestyle and treatment related predictors of LBM after completion of therapy for breast cancer. This will be done to further elucidate the role of modifiable risk factors for adverse body composition change following treatment

Primary Hypothesis

Breast cancer survivors participating in a specifically designed group based cognitive behaviour therapy nutrition and exercise program and supplementing with 3g LCn-3s will have greater attenuation of LBM loss after 12 weeks compared to participants taking a supplement of 3g LCn-3s alone.

Secondary Hypotheses

Breast cancer survivors participating in the group based cognitive behaviour therapy nutrition and exercise program taking a supplement of 3g LCn-3s will have improved quality of life after 12 and 24 weeks compared to participants of either the specifically designed nutrition and exercise

program or participants taking a supplement of 3g LCn-3s alone.

Breast cancer survivors in the group based cognitive behaviour therapy nutrition and exercise program taking a supplement of 3g LCn-3s acids will have lower levels of inflammation after 12 and 24 weeks compared to participants of either the specifically designed nutrition and exercise program or participants taking a supplement of 3g LCn-3s alone.

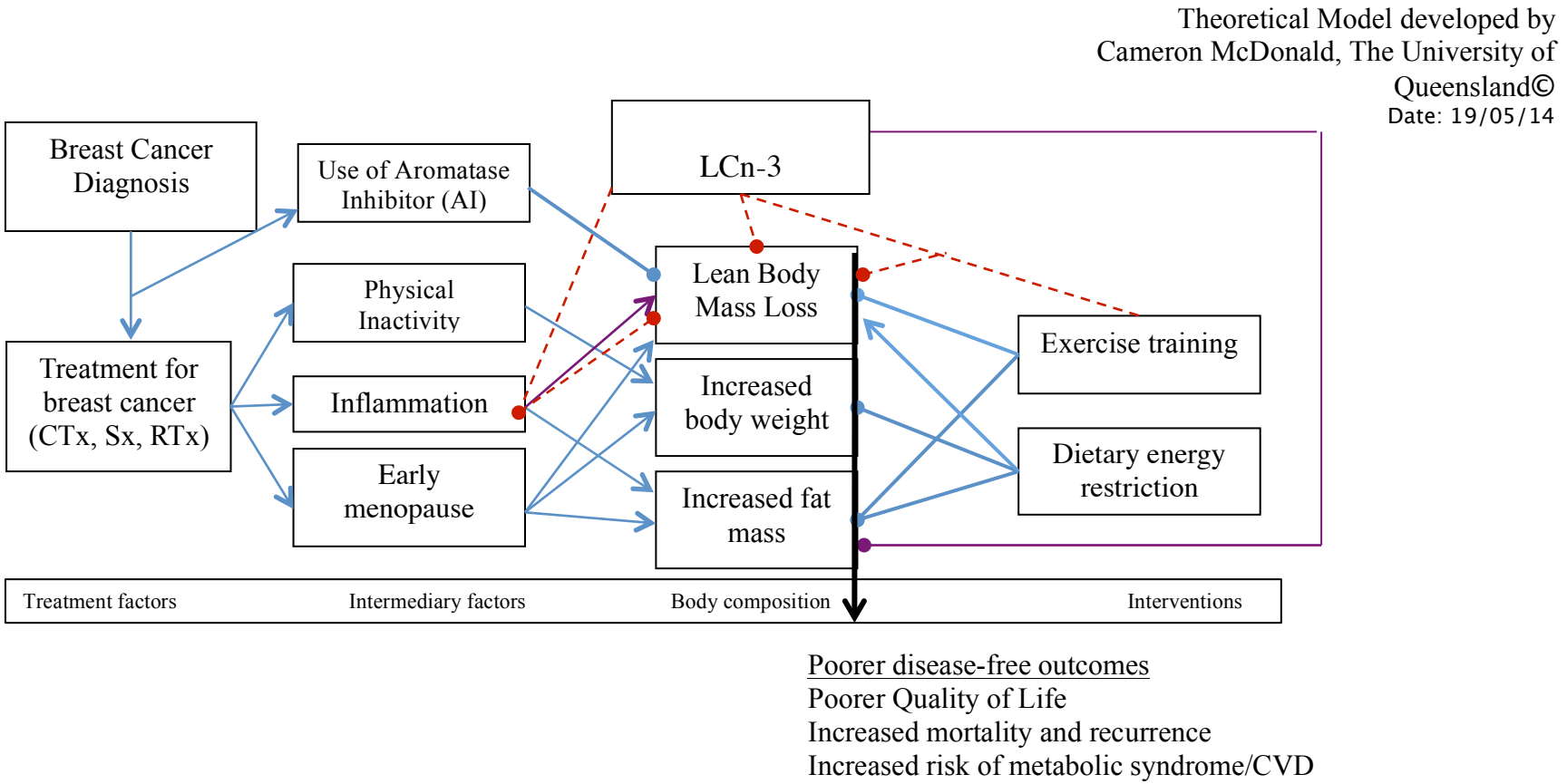


Figure 2.1 Theoretical model of factors influencing body composition change after treatment for breast cancer

Treatment factors: After a diagnosis of breast cancer, treatment of the cancer (CTx: Chemotherapy; Sx: Surgery; RTx: Radiation therapy) results in a number of adverse behavioural and physiological changes, which negatively affect body composition in the three to four years following treatment (Demark-Wahnefried, Campbell, and Hayes 2012).Intermediary factors: Treatment with Aromatase Inhibitors are associated with increments in LBM over time (Francini et al. 2006, Montagnani, Gonnelli, et al. 2008). Studies in breast cancer populations indicated by a blue line — (an arrow → indicates agonistic effect; solid circle • indicates antagonistic effect) show a reduction in physical activity has been associated with increased adiposity(Irwin et al. 2005), while treatment related menopausal changes have been shown to adversely affect LBM, body fat and body weight (Goodwin et al. 1999, Demark-Wahnefried et al. 2001). Inflammation as measured by CRP has been associated with greater fat mass(Dee et al. 2012). A review of research in non-breast cancer populations (purple line —) indicates that increased inflammation is associated with LBM loss and increased fat mass (Mourtzakis and Bedbrook 2009). Evidence indicates these adverse changes are associated with poorer disease-free survival. Interventions: Exercise training has been shown to increase LBM(Schmitz, Ahmed, et al. 2005, Herrero et al. 2006), decrease adiposity(Irwin, Alvarez-Reeves, et al. 2009, Kim, Kang, and Park 2009), while dietary energy restriction has reduced total and fat mass(Thomson et al. 2010). However, dietary energy restriction alone has also been shown to adversely decrease LBM(Thomson et al. 2010).Omega-3 fatty acids (LCn-3) have been shown to prevent loss of LBM in NSCLC patients (Murphy, Mourtzakis, and Mazurak 2012).yet not in non-cancer populations; previous reports indicate reduced body weight and adiposity in LCn-3 supplemented groups(Kabir et al. 2007, Noreen et al. 2010, Couet et al. 1997, Hill et al. 2007). It is currently unknown in breast cancer survivors (red dashed line --) if LCn-3 supplementation can protect LBM alone, in conjunction with a diet and exercise program, and if it does, whether this is a) modified through a decrease in inflammation, and b) more effective than the diet and exercise program alone. (LBM: Lean body mass)

Chapter 3 – Methods

3.1 Overview

The primary aim of this study was to compare the effect of LCn-3 supplementation alone (N-3), a combination of a specialised exercise and nutrition lifestyle program plus LCn-3 (Ex+N-3), and the lifestyle program plus olive oil (EP+OO), on changes in LBM in women who had completed treatment for breast cancer in the last 12 months. Secondary outcomes included changes in QOL, inflammation, other components of body composition and LBM function variables. Subjects were randomly allocated to one of the three groups for six months following baseline assessment; they were then re-assessed at 12 and 24 weeks after baseline.

The first section of this chapter is presented as a published manuscript. It describes the study protocol with a focus on the primary outcome measures and a small number of secondary outcome measures. The manuscript outlines the relevant literature for our study design, which is a condensed repeat of the previous chapter. The methods section of the manuscript describes the assessment procedure for the primary and secondary outcome measures. In addition, the manuscript provides a list of auxiliary measures, which are further described in the second section of the chapter. Finally, power calculations and statistical analyses are outlined in the manuscript

The second section of the chapter provides more complete information on the justification and procedures for the auxiliary outcome measures. Considerations for the inclusion of outcome measures and accuracy are described in this.

3.2 Published Manuscript #3:

The Muscle mass, Omega-3, Diet, Exercise and Lifestyle (MODEL) study – a randomised controlled trial for women who have completed breast cancer treatment

BMC Cancer

STUDY PROTOCOL Open Access

The muscle mass, omega-3, diet, exercise and lifestyle (MODEL) study – a randomised controlled trial for women who have completed breast cancer treatment

Cameron McDonald[1*], Judy Bauer[1], Sandra Capra[1] and Joseph Coll[2]

Abstract

Background: Loss of lean body mass (LBM) is a common occurrence after treatment for breast cancer and is related to deleterious metabolic health outcomes [*Clin Oncol*, **22**(4):281–288, 2010; *Appl Physiol Nutr Metab*, **34**(5):950–956, 2009]. The aim of this research is to determine the effectiveness of long chain omega-3 fatty acids (LCn-3s) and exercise training alone, or in combination, in addressing LBM loss in breast cancer survivors.

Methods/design: A total of 153 women who have completed treatment for breast cancer in the last 12 months, with a Body Mass Index (BMI) of 20 to 35 kg/m^2, will be randomly assigned to one of 3 groups: 3g/d LCn-3s (N-3), a 12-week nutrition and exercise education program plus olive oil (P-LC) or the education program plus LCn-3s (EX+N-3). Participants randomised to the education groups will be blinded to treatment, and will receive either olive oil placebo (OO+N-3) or LCn-3 provision, while the N-3 group will be open label. The education program includes nine 60-75min sessions over 12 weeks that will involve breast cancer specific healthy eating advice, plus a supervised exercise session run as a resistance exercise circuit. They will also be advised to conduct the resistance training and aerobic training 5 to 7 days per week collectively. Outcome measures will be taken at baseline, 12-weeks and 24-weeks. The primary outcome is % change in LBM as measured by the air displacement plethysmograhy. Secondary outcomes include quality of life (FACT-B + 4) and inflammation (C-Reactive protein: CRP). Additional measures taken will be erythrocyte fatty acid analysis, fatigue, physical activity, menopausal symptoms, dietary intake, joint pain and function indices.

Discussion: This research will provide the first insight into the efficacy of LCn-3s alone or in combination with exercise in breast cancer survivors with regards to LBM and quality of life. In addition, this study is designed to improve evidence-based dietetic practice, and how specific dietary prescription may link with appropriate exercise interventions.

Trials registration: ACTRN12610001005044; and World Health Organisation Universal trial number: U1111-1116-8520.

Keywords: Breast cancer, Omega-3 fatty acids, Body composition, Exercise, Lean body mass, Inflammation

Background

Breast cancer is the predominant cancer diagnosed in women with 1.4 million new cases diagnosed worldwide in 2008 [1]. Modern treatment protocols have resulted in a 5-year survival rate of 85% to 90% in developed countries, with Australia's reported at 89.4% in 2012 [2]. Following treatment for breast cancer, a majority of women experience significant body weight increases [3-5]. These changes unfortunately, are comprised of simultaneous lean body mass (LBM) loss and fat tissue gain [4-7]. Furthermore, LBM loss and fat mass gains have been shown to occur in the absence of total body weight change [8]. Data from breast cancer cohorts reveal that weight gain is most strongly associated with premenopausal status at diagnosis [4], those who experience menopause as a result of treatment [4,9], lower weight at diagnosis, lower levels of physical activity [10], and longer chemotherapy treatment [5]. Evidence from pharmacological trials indicate that initial use of [11], or switching to aromatase inhibitors from

* Correspondence: c.mcdonald4@uq.edu.au
[1]Centre for Dietetics Research, University of Queensland, Brisbane, Australia
Full list of author information is available at the end of the article

tamoxifen [12,13] increases LBM, possibly due to the alteration in sex steroid balance. The complete aetiology of general LBM loss in this population is unclear, however it appears to be associated with poorer metabolic outcomes, such as earlier onset of cardiovascular disease and metabolic syndrome related diseases [14,15].

Currently, no definitive recommendations can be made in regards to the ideal weight or weight change for women who have completed treatment for breast cancer. Epidemiological studies using weight or BMI have indicated that weight stability may confer benefits in terms of mortality [16-18]. Currently there have been no trials assessing mortality and the impact of body composition change (LBM and fat tissue), however results from shorter intervention trials indicate that intentional weight loss and increased activity can improve biochemical markers associated with cardiovascular disease [19-21] and conditions related to metabolic syndrome [19,21], which both account for significant morbidity and mortality in this population.

Interventions to improve body composition in women diagnosed with breast cancer

A number of studies have assessed the impact of diet, exercise or combined therapies on body composition during or following treatment reporting mixed effects. From studies that have reported a high quality measure of body composition assessment (i.e. Dual-Energy X-ray Absorptiometry: DEXA; Air Displacement Plethysmography: ADP; Computed Tomography: CT-scan; and Magnetic Resonance Imaging: MRI) resistance training is most likely to cause an increase in LBM [22,23], aerobic training overall has had mixed effects on LBM [21,24-26], with most studies indicating no change. Two studies that prescribed a combination of resistance and aerobic training have shown an increase in LBM [27,28]. Considering that aerobic exercise has been associated with improved disease-free survival in breast cancer populations [29,30], a combination of resistance and aerobic two may promote LBM growth and survival benefits extending beyond the study timeline. Some data indicate LBM increases may be more likely in younger individuals, and separately, those taking aromatase inhibitors (AIs) [21,31]. Dietary energy restriction alone has resulted in significant body weight loss but also involves significant LBM loss [19], while combining nutrition and exercise prescription may help to preserve LBM during weight loss [32], and/or ameliorate fat tissue gain during weight stability [33].

Exercise and nutrition trials during chemotherapy

Numerous uncontrolled and controlled trials have been conducted assessing change in body weight and/or body composition. Of these trials, 11 studies that have used a high quality measure of body composition have indicated mixed effects on lean body mass for different modalities. Exercise only interventions conducted during chemotherapy have indicated that resistance exercise training is probably required to realise an increase in LBM [22], while aerobic training alone has shown little to no impact on LBM change [26]. When Courneya et al (2007) confined their analysis to women with more advanced breast cancer (Stage IIb & IIIa) significant improvements were seen in the intervention group compared to control, these differences were not seen in those women with earlier stage disease (Stage 0-IIa) [31]. Comparatively, combined exercise and nutrition interventions during chemotherapy have typically shown no effect on LBM change [33-35]. Lack of LBM gains may be a result of the less intensive/structured exercise training components prescribed in combined trials.

Exercise and nutrition trials after completion of chemotherapy

A larger literature exists describing effects of exercise and nutrition on LBM in women after they have completed treatment (up to 3 to 4 years post). Of the four [21,23-25] studies reporting a high quality measure of body composition after exercise alone, two aerobic exercise studies (one controlled, one uncontrolled) reported statistically non-significant trends in LBM change [24,25], while separate aerobic [21] and resistance training [23] trials indicated a significant increase in LBM compared to control groups (+0.8 kg vs -0.8 kg, $p = 0.047$ & +0.88 kg vs +0.02 kg, $P = 0.008$, respectively). After further analysis, Irwin et al [36] found that exercisers aged <56 years had greater LBM gains than women >56 years and non-exercisers, and those taking AIs and exercising had greater LBM increases than those not taking AIs.

One well-designed study investigated dietary energy restriction alone on body composition and examined the differing effects of a low energy and low fat intake or low energy and low carbohydrate intake [19]. Both groups lost a similar and significant amount of body weight (6.1 kg + 4.8 kg) over 12 months, unfortunately this body weight change occurred at the expense of fat tissue and LBM. Incidence of sarcopenia, as defined by an appendicular LBM of <5.67 kg/m^2, increased from 10% at baseline to 18% at the end of the trial [19,37].

Of the two studies that have assessed the effect of exercise and nutrition combined on LBM, one study has shown that LBM may be preserved by exercise during dietary energy restriction [32], while the other indicated a reduction in fat accumulation with no change to LBM during dietary energy balance [32,33,38]. After a 2000-4000kJ energy restriction plus a combined aerobic and resistance training protocol, Mefferd et al (2007) noted stable LBM in both intervention and wait-list control groups, however compared to control, the intervention

group had a significant reduction in total body weight (-0.5 kg vs. -5.7 kg, $p < 0.05$) [32]. Preservation of LBM during significant weight loss could be viewed as a positive outcome in this population as losses of LBM are typical. In a later study that did not use an energy restriction, Demark-Wahnefried et al (2008) [33] assessed the effects of calcium rich diet alone (1200-1500 mg/day), combined with low load resistance training (30 min, 3/wk), or combined with the exercise and a low-fat, high fruit and vegetable intake. No change over time in LBM was seen within or between groups, however when trunk fat was excluded from calculations, the third group experienced less body fat % gain over the 6 month intervention than the other two groups (Change in body fat%: Gp3: +0.2% vs Gp1: +1.7% & Gp2: +1.1%, respectively, $p < 0.05$). The lack of LBM change is most likely due to the low frequency and low-load callisthenic type resistance training prescribed, which may not have been adequate for optimal stimulation of muscle protein synthesis.

Taken together, LBM loss is most likely prevented by resistance training in women who have been or are being treated for breast cancer. Increases in LBM may be confined to those women who adhere to more intensive exercise protocols [21-23], or in specific sub-populations related to younger age [21] or later stage disease [31]. Dietary energy restriction alone at this stage could be considered contraindicated due to the heightened risk of sarcopenia in this population, while the addition of exercise to an energy restriction may ameliorate this risk [32]. At this stage, no studies have aimed to combine dietary prescription and exercise training to specifically increase LBM. Amino acids and long chain omega-3 fatty acids (LCn-3 FAs) are two potential nutrients that can be targeted to compliment resistance training, yet data is lacking in breast cancer survivor populations.

Advances in nutritional supplementation and support for exercise training in other populations indicate that inclusion of specific nutrients, such as amino acids [39] or possibly long chain omega-3 fatty acids (LCn-3s) [40], may significantly enhance the response of LBM in conjunction with exercise training. To date, studies using dietary interventions in breast cancer have not utilised either of these nutrients to improve LBM outcomes for survivors.

Omega-3 and body composition change

LCn-3s have been extensively investigated for their ability to preserve LBM in other cancer populations [41,42]. However, the populations typically studied have been those with metastatic or advanced cancer and cachexia. Breast cancer survivors do not experience LBM losses comparable to cachectic populaionts, they are much more like a metabolic syndrome population who undergo slower change often associated with fat gains [43].

Long chain omega-3 fatty acids have been considered as potential body composition modulators with or without dietary energy restriction [44]. However, due to significant heterogeneity in population, body composition measurement, length of trial and dose of LCn-3s some trials have reported no effect [45-50], while others have indicated some effect [51-54]. However, of the studies reporting an improvement of one of more body composition parameters after increased LCn-3s intake, the clinical significance of the changes in LBM seen are minimal [40].

In contrast, recently published data indicate that LCn-3s may have clinical utility as an adjunct to an anabolic stimulus like resistance training [55] or during a hyperaminoacidaemic/hyperinsulinaemic clamp [56,57]. Preliminary evidence suggests that LCn-3s may have a permissive effect on muscle protein synthesis, i.e. reducing anabolic resistance [56,57], and may improve neural activation [55] such that skeletal muscle tissue exhibits a greater response to a given anabolic stimulus. In addition, LCn-3s were seen to improve the 'anabolic resistance' found in older populations [56].

The safety of LCn-3 supplementation for doses of up to 4g of EPA & DHA/day has been established as low, with the most common concerns arising in regards to gastrointestinal upset and allergic reactions [58]. There is a theoretical link to an increased risk of bleeding when taken in conjunction with anti-coagulant medication, however this is not considered to be a contraindication in these populations [59].

To the authors' knowledge, no studies have assessed the effect of exercise training and LCn-3 supplementation alone or together, in women who have had breast cancer. Therefore the current study is aimed at comparing the effects of LCn-3FAs alone, an exercise and nutrition program alone, or a combination of both, and how they influence LBM, QOL and inflammation over 12 and 24 weeks in women who have recently completed treatment for breast cancer. It is hypothesised that the greatest relative LBM gains will occur in the combination group.

Methods/design

Primary hypothesis

Breast cancer survivors participating in a specifically designed group based cognitive behaviour therapy nutrition and exercise program and supplementing with 3g LCn-3s will have greater attenuation of LBM after 12 weeks compared to participants taking a supplement of 3g LCn-3s alone.

Secondary hypotheses

Breast cancer survivors participating in the group based cognitive behaviour therapy nutrition and exercise program taking a supplement of 3g LCn-3s will have improved

quality of life after 12 and 24 weeks compared to participants of either the specifically designed nutrition and exercise program or participants taking a supplement of 3g LCn-3s alone.

Breast cancer survivors in the group based cognitive behaviour therapy nutrition and exercise program taking a supplement of 3g LCn-3s acids will have lower levels of inflammation after 12 and 24 weeks compared to participants of either the specifically designed nutrition and exercise program or participants taking a supplement of 3g LCn-3s alone.

Trial design

In order to determine the relative efficacy of each intervention, the design of the study is a parallel 3-arm randomised controlled trial. The intervention will occur at one site, with recruitment occurring at multiple sites. The primary investigators and the participants allocated to the exercise and nutrition groups (+/- LCn-3s) will be blinded, while the LCn-3 FAs alone group is open label.

Details of power calculation and sample size

The primary outcome measure is change in lean body mass (LBM) at 12 weeks. Exercise interventions In breast cancer populations have shown LBM increases of 0.7 kg to 1 kg [21-23,27], however other exercise intervention studies have reported attenuation of LBM loss rather than increase [60-62]. Assuming that the minimum difference in LBM across the comparison groups is a mean of 2%, 38 participants per group will be required to detect this difference with 90% power and type 1 error of 5% or less (two-tailed) A total of 114 participants are therefore required. Assuming 10% for attrition and allowing 15% for contingency, 51 subjects per group will need to be recruited to obtain complete data on at least 38 for each group.

The study is sufficiently powered to test the secondary hypotheses. A Bonferroni correction to the Type I error will accommodate the 3 pair-wise comparisons by 2 visits such that $p < 0.008$ will be considered statistically significant in order to preserve the family-wise Type I error rate of 5% for each secondary outcome.

Participant recruitment

Women will be recruited through breast cancer oncology centres, radio advertising, social media and breast cancer research registries in Brisbane, Australia. Oncologists, breast care nurses and allied health professionals will inform potential participants of the study during or shortly following treatment (surgery, radiotherapy and/or chemotherapy). Participants will be asked to contact the primary investigator to express official interest in the study and have eligibility determined. Recruitment into the trial will be over 10 to 15 groups of 5 to 15 participants per group. A range of group sizes has been chosen to ensure the maximum number of participants can be recruited as delaying the start of intervention may result in some being excluded due to time elapse since treatment. Within each of these groups, participants will be randomly allocated to one of 3 groups. A record will be kept of the number of participants who have expressed interest to the primary investigator, the number of potential participants who are eligible, ineligible and then finally randomised into the trial. Ethical approval has been received from the participating hospital (UCH HREC: #1034) as well as from the University of Queensland (#2011000079). All participants will provide written informed consent.

Eligibility criteria

To be included in the study, the women must be >18 years of age; have been diagnosed with early stage breast cancer (Stage 0-IIIa); have successfully completed surgery, radiotherapy and/or chemotherapy more than 6 weeks prior to allow for wound healing and/or shoulder recovery, but not more than12 months post completion of treatment (participants can be currently receiving endocrine and/or herceptin therapy); able to perform moderate intensity physical activity, and have a BMI of >20 and <35 kg/m^2.

Participants will be excluded if: they have completed their treatment more than 12 months ago; there is presence of metastatic growth or local/distal recurrence of cancer; they have been diagnosed with cardiovascular disease or diabetes; they currently consume, or have in the last 3 months been consuming >1 g of eicosapentanoic acid (EPA) and docosahexanoic acid (DHA) LCn-3s combined per day; or they refuse to be randomly allocated to one of the 3 groups.

Those who are ineligible will be referred to their general medical practitioner with encouragement to pursue appropriate lifestyle recommendations.

Randomisation

The supplier of the capsules who has no direct contact with the participants will use NQuery Version 7 mixed block design to randomise group order. Participants will be allocated to their group in the order in which they complete baseline assessment. This trial has been registered with Australia New Zealand Clinical Trials Registry: ACTRN12610001005044; and World Health Organisation: Universal trial number is U1111-1116-8520.

Interventions

The 3 intervention arms include: Daily consumption of LCn-3 FAs (N-3) for 24 weeks; Daily consumption of LCn-3 FAs for 24 weeks plus a supervised 12-week exercise and nutrition group education program (EX+N-3);

Daily consumption of placebo oil for 24 weeks and the 12-week program (OO+N-3).

Long chain omega-3 fatty acid supplementation

Both N-3 and EX+N-3 groups will be prescribed 3 g (1.75 g EPA and 1.25 g DHA) per day taken in five 1 g capsules each containing 0.35 g and 0.25 g for EPA and DHA, respectively. Participants will be recommended to take the dose with a meal, either all at once or spaced throughout the day. Refrigeration of the capsules will be also recommended.

Placebo supplementation

The OO+N-3 group will be prescribed five 1 g capsules containing olive oil. The placebo capsules are visually identical to the LCn-3 capsules and created by the same vendor.

All capsules were created in the same batch and were sample tested to ensure they contained the indicated dose. All Participants will be asked to avoid ongoing supplementation of any source that contains additional LCn-3s.

Exercise and nutrition education program

EX+N-3 and OO+N-3 groups will be asked to attend 9 nutrition and exercise sessions over 12 weeks, starting 1 to 10 days after baseline assessment. To ensure adequate group size, both EX+N-3 and OO+N-3 groups will participate in the same sessions. Both participants and primary investigator will be blinded to group allocations, while all capsules will be given out separately to minimise product comparison. The sessions will run for 60-75 minutes at the Wesley Research Institute, Brisbane. The sessions will include 30 to 45 minutes of nutrition education, the remainder of the time is committed to resistance exercise training. The sessions will be facilitated by the Primary Investigator who is an Accredited Practising Dietitian and Accredited Exercise physiologist with relevant clinical experience.

Semi-supervised exercise program

The supervised exercise sessions are designed as circuit based training sessions. The sessions will be started with active range of motion exercises as a warm up, exercises will then performed and the session completed with specific stretches and flexibility exercises. The exercises include push ups, squats*, lunges, glute bridging, seated row*, shoulder press*, bicep curls* and a series of postural and abdominal exercises (*Exercises marked indicates the use of the Gymstick™). The resistance exercise program is designed to be performed at home using body weight and the Gymstick™, a specialised elastic resistance stick, which has been used in a previous non-cancer population of similarly aged participants [63]. During the supervised sessions feedback will be given regarding technique, exercise progression and modification, and management of injury/discomfort. Participants will be prescribed to reach at least 3 resistance sessions per week including the supervised session, and at least 3 aerobic training sessions each week at home. The participants will be given access to specifically made video material that details the appropriate technique for the majority of the exercises performed in class. The program will be progressed with the addition of new exercises, increased difficulty of exercises by increasing the tension of the Gymstick™, or exercise modification, and through an increase in workload volume (repetitions and sets). Typically, each exercise will be performed as many times as possible in 30-second to one-minute bouts, or until temporary fatigue. This type of workload has been chosen as it is most applicable to home training using body weight and elastic apparatus. In addition, research indicates that reaching temporary fatigue through a low load high-volume protocol results in a similar increase in muscle protein synthesis when compared to a high load protocol with less repetitions [64].

Nutrition and exercise education program

The nutrition education program was based on a previously validated cognitive behavioural program for weight loss [65] and adapted to focus on healthy food choices for breast cancer survivors. It should be noted that participants will not be given additional advice regarding weight loss or energy restriction throughout the trial. The 9 sessions will include advice on general and breast cancer specific healthy eating, benefits of exercise and practicalities of incorporating healthy habits. Group discussion will be facilitated by the primary investigator to increase practical content. All of the nutrition sessions will be recorded on the Powerpoint slides and provided to the group members via an online portal.

Side effects of treatment

All participants will be asked to report the appearance of any adverse symptoms that may be related to the exercise program or capsule consumption. If a participant is diagnosed with a recurrence they will be excluded from the data analysis. They will also be advised in how to access ongoing lifestyle treatment in a private setting. If participants report an exacerbation of lymphoedema symptoms they will be referred to a breast cancer specialist physiotherapist for assessment, in addition, they will be advised to cease their upper body resistance training until medical clearance is given to continue as per the ACSM guidelines [66]. For gastro-intestinal upset, or unpredicted reactions that arise during the study period, participants will be asked to cease capsule consumption and advised to seek medical clearance before recommencing.

Measures

All outcome measures will be performed at baseline, 12 and 24 week time points. The 24 week time point has been included to better understand the practicality of the intervention in terms of maintenance of lifestyle changes after the supervised time. Each assessment period will involve 2 visits to the WRI. Visit 1 measures will include body composition, questionnaires and aerobic fitness testing. Over the next 7 days participants will be asked to: complete the Diet History Questionnaire; wear a uniaxial accelerometer every day; and have a fasting blood sample. At Visit 2, the primary investigator will review the diet history questionnaire, collect the accelerometer and conduct the muscle endurance testing. The progression of participants through the study can be seen in Table 1, with specific timing of outcome measures shown in Table 1.

Primary outcome measure

Body composition

Change over time in percentage lean body mass will be measured using air displacement plethysmograhy (ADP) (BODPOD, COSMED USA Inc). Before each assessment day, the BODPOD scales and air chamber is calibrated as per the manufacturer's instructions using known weights and volumes, respectively. Air Displacement Plethysmography is considered a valid alternative to hydrodenitometry (or underwater weighing); it is based on the two-compartment model which views the body as two distinct chemical components composed of FM and FFM [67]. ADP was validated against hydrostatic weighting and generated similar result with good precisions when tested repeatedly [67,68]. Amongst health subjects, ADP has been shown to agree well with other laboratory methods including DXA [69,70] and isotope dilution [71]. All measures will be performed by a certified BODPOD assessor.

Participants will be assessed in a non-fasted state. To minimise daily weight variations, participants are measured at a similar time of day (within 60 minutes of initial assessment) at all 3 assessment points. Consumption related weight variations will be controlled by a food and drink record. At the 12 and 24 week assessments, participants will be asked to repeat their intake from the initial assessment. Participants will be provided a lycra suit and hair cap designed for the BODPOD that must be worn during the assessment. Weight is measured with the electronic scale attached to the BODPOD system. Height is measured using a wall mounted stadiometer. The predicted thoracic volume generated by BODPOD software is used for all calculations.

Secondary outcome measures

Quality of life (QOL)

QOL will be measured using the Functional Assessment of Cancer Therapy- Breast + 4 (FACT-B + 4) tool. This tool has been validated for quality of life measurement in cancer survivor populations [72], breast cancer treatment-related arm morbidity [73], measuring QOL change following exercise training [74], and is one of the most widely cited tools in breast cancer research [75] It is comprised of 2 separate tools, the 27-item FACT-G, and the additional 14-item 'B + 4' that specifically relates to individuals who have been treated for breast cancer. A five-point Likert scale is utilised (ranging from 0 = 'not at all' to 4 = 'very much') and includes four subscales (physical, social, emotional, and functional well-being). Higher scores represent better well-being.

Table 1 Timings for baseline, 12 & 24 week assessments

	Day 1 – Approx 2 hours	Days 2-6	Day 7 – approx 15 mins
Baseline assessment	- Consent form and eligibility assessed	- Fasting CRP and EFA test	- Hand in Accel
Week -1 to 0			- Accel
	- LBM,		- Squat
	- Body fat%, Wt, Ht, Waist, Hip		- Push up
	- QOL related questionnaires		- DHQ
	- TMill		
	- Handgrip strength		
	- Demographical info		
	- Accel given		
Mid intervention assessment		As above except for consent form + Pill counts	
Week 12-13			
Post-intervention assessment		Same as mid-intervention assessment	
Week 25-26			

LBM: Lean body mass; Wt: Weight & Ht: Height; Waist & Hip: Girths; QOL: Quality of Life; TMill: Treadmill Sub-max Vo2 test; Accel: 7-day accelerometer; DHQ: Dietary Habits Questionnaire; CRP: Fasting high sensitivity C-reactive protein; LCn-3: Fasting erythrocyte fatty acid analysis; Push up: 60-second push up test; Squat: 60-second squat test.

C-reactive protein

A fasting high sensitivity-CRP will be measured using a latex-enhanced immunoturbidimetric assay of blood serum. Participants will be asked to attend a Healthscope Pathology lab between Day 1 and 7 of each respective assessment period to have a fasted blood sample taken by a qualified lab technician.

Body composition

Percentage and total body weight, adipose tissue content will be measured using the BodPod as described above.

Measure of adherence to capsule intake

Long chain omega-3 fatty acid intake will be accounted for in two ways: erythrocyte LCn-3 FA content, and combination of pill count and diet history questionnaire.

Erythrocyte fatty acid analysis

Lipids from red cells are extracted with chloroform methanol mixture. The fatty acids are trans-esterificated to methyl esters with methylation reagent "Meth-Prep 2". The methylation extract is analysed by gas liquid chromatography method with flame ionisation detection (gas chromatograph Schimadzu G-2010-FID). The proportion of fatty acids content of the erythrocytes expressed as % of total fatty acids.

Pill count

All capsule bottles will be handed in at the end of each 12 weeks. All pills not consumed will be counted and recorded over the 24 weeks.

Measure of adherence to exercise and dietary program

The Active Australia Survey [76], 7-day Uniaxial accelerometry and exercise log during the intervention will be completed for all assessment points in order to determine changes to physical activity. In addition, changes in push-ups and squats will be considered an indirect marker of exercise adherence. Dietary intake will be assessed by an Accredited Practising Dietitian using the Dietary Habits Questionnaire [77]. Additionally, attendance at sessions will be recorded for each group.

A number of other measures will be taken to capture changes in sub-maximal aerobic fitness [78], upper body strength-endurance [78], lower body strength-endurance [78], handgrip strength [79], waist and hip girths [80], fatigue [81], physical function [82,83] and menopausal symptoms [84]. The tools to be used to measure the above are shown in Table 2.

Table 2 Additional measures taken at baseline, 12-weeks & 24 weeks

Outcome	Tool
Physical activity & sedentary time	-7-day uniaxial accelerometery
	-Active Australia Questionairre
	-Training log book (Wk 12 & 24)
Changes in aerobic fitness	-Sub-maximal treadmill test (modified Balke)
Muscular endurance	Upper body: 1-min-push up test
	Lower body: 1-min sit-to-stand test
Muscle strength	Grip strength
Dietary intake	Diet history questionnaire
Waist and hip girth	Metal tape measure
Joint pain and physical function	Health Assessment Questionnaire – Disease Index (HAQ-DI)
Menopausal symptoms	Greene Climacteric Scale

Data analysis

The primary analysis population is intention to treat. The ITT population will include all randomised participants with at least one post-baseline assessment. Analysis will also be performed on the per protocol (PP) population. The PP population will include all participants who were at least 75% compliant to the exercise and nutrition program (as measured by the number of exercise sessions attended) and 70% adherent to pill intake (as measured by pill count returned/diet history questionnaire or by erythrocyte LCn-3 FA).

Baseline demographic and disease related characteristics will be summarised by group as count and percent for categorical variables and number, mean and standard deviation for continuous variables. To compare the three treatment groups at baseline, a chi square test or Fishers exact test will be used for categorical variables and a one way analysis of variance (ANOVA) or Wilcoxon rank-sum test for continuous variables. Baseline demographic and disease-specific characteristics that differ among groups will be considered for covariate adjustments in analysis of all outcomes.

Measurements collected longitudinally will be summarised by group as number, mean and standard deviation, minimum and maximum at each visit (baseline, 12 week, 24 week). Absolute change from baseline will be calculated by subtracting the baseline measurement from the 12 week and 24 week measurements; percent change from baseline will be calculated by dividing the 12 week and 24 week measurements by the baseline measurement. Measurements include: body composition, quality of life, C-Reactive Protein, physical activity and sedentary time, aerobic fitness, muscular endurance, muscle strength, dietary intake, waist and hip girth, joint pain and physical function and menopausal symptoms. All outcome data will be visually inspected for normality. Data with a skewed distribution may be transformed (e.g. log transformation).

The primary outcome of percent change in lean body mass at 12 weeks between the N-3 and EX+N-3 groups will be tested using a contrast in a one-way ANOVA and a p-value <0.05 will be considered statistically significant. Secondary outcomes will be evaluated using mixed models to accommodate the correlation of the repeated measurements taken on an individual over time. For each of the change from baseline outcomes, time (12 week vs 24 week), treatment (N-3, EX+N-3, OO+N-3), the time by treatment interaction and the baseline value of the outcome will be tested as fixed effects with a random subject effect specified. Interactions with $p < 0.10$ will be retained in the models. Contrasts will be constructed to compare pair-wise differences among the three treatment groups at each time point. Similar mixed models will be fit to evaluate the effect of adjusting for covariates. In addition to the fixed effects for time, treatment, time by treatment and baseline value, covariates at baseline identified as statistically different among the three groups and covariates known or hypothesised to be associated with the particular change from baseline outcome will be evaluated as fixed effects.

Discussion

This study will further the evidence base in regards to omega-3 and exercise synergies. These findings will be applicable to breast cancer populations and may translate to populations with other chronic diseases. The cessation of LBM loss, fat mass gain and the associated metabolic benefits are an important consideration for women after breast cancer treatment. Thus, the applicability of known and practical lifestyle measures is an important consideration for ongoing management.

Abbreviation

ADP: Air displacement plethysmography; BMI: Body mass index; CT-scan: Computed tomography; Demo: Demographics; DEXA: Dual-energy X-ray absorptiometry; E-LC: Education program plus LCn-3s; FACT-B + 4: Functional assessment cancer therapy – breast +4 items; Hs-CRP: High sensitivity C-reactive protein; LBM: Lean body mass; LC: 3g/d LCn-3s alone; LCn-3 FAs: Long chain omega-3 fatty acids; MRI: Magnetic resonance imaging; P-LC: 12-week nutrition and exercise education program plus olive oil group; QOL: Quality of life.

Competing interests

The author's declare that they have no competing interests.

Authors' contributions

CM contributed involved with project design and ethical approval. CM is responsible for overall project management: recruitment, data collection and intervention delivery. JB and SC conceived and sought funding for the trial. JB contributed to study logistics, expertise related to LCn-3 measurement, and intellectually assists with trial management. SC has ongoing intellectual input into research protocol and data analysis. JC provided expertise in regards to statistical analysis and consistency of outcome measure administration and data collection. All authors read and approved the final manuscript.

Authors' information

Primary investigator: Cameron McDonald; Accredited Practising Dietitian, Accredited Exercise Physiologist.

Acknowledgements

Funding for the study was put forward by the Wesley Research Institute, this funding covered blood analyses, purchase of necessary equipment and payment for research assistance when required. The authors acknowledge that all capsules were provided *gratus* by Blackmores Ltd, Australia. In addition, GymStick provided exercise equipment for all participants at cost price. Parties associated with the supply of capsules and exercise equipment have no part in the research design, administration, analyses and subsequent publications.

Author details

[1]Centre for Dietetics Research, University of Queensland, Brisbane, Australia. [2]The Wesley Research Institute, Brisbane, Queensland, Australia.

Received: 21 January 2013 Accepted: 26 March 2014
Published: 16 April 2014

References

1. Youlden DR, Cramb SM, Dunn NAM, Muller JM, Pyke CM, Baade PD: **The descriptive epidemiology of female breast cancer: An international comparison of screening, incidence, survival and mortality.** *Cancer Epidemiol* 2012, **36**(3):237–248.
2. Australian Institute of Health and Welfare & Cancer Australia: Breast cancer in Australia: an overview. Cancer series no 71: *Cat. no. CAN 67*. Canberra: AIHW; 2012.
3. Sheean PM, Hoskins K, Stolley M: **Body composition changes in females treated for breast cancer: a review of the evidence.** *Breast Cancer Res Treat* 2012, **135**(3):663–680.
4. Demark-Wahnefried W, Peterson BL, Winer EP, Marks L, Aziz N, Marcom PK, Blackwell K, Rimer BK: **Changes in Weight, Body Composition, and Factors Influencing Energy Balance Among Premenopausal Breast Cancer Patients Receiving Adjuvant Chemotherapy.** *J Clin Oncol* 2001, **19:**2381–2389.
5. Harvie M: **The Importance of Controlling Body Weight After a Diagnosis of Breast Cancer: The Role of Diet and Exercise in Breast Cancer Patient Management.** In *Book title: Exercise and Cancer Survivorship.* 2010:73–96.
6. Rooney M, Wald A: **Interventions for the Management of Weight and Body Composition Changes in Women With Breast Cancer.** *Clin J Oncol Nurs* 2007, **11**(1):41–52.
7. McDonald CK, Bauer JD, Capra S: **Body Composition and Breast Cancer-the Role of Lean Body Mass.** *Cancer Forum* 2011, **35**(2):102–106.
8. Kutynec CI, McCargar L, Barr SI, Hislop TG: **Energy balance in women with breast cancer during adjuvant treatment.** *J Am Diet Assoc* 1999, **99:**1222–1227.
9. Goodwin PJ, Ennis M, Pritchard KI, McCready D, Koo J, Sidlofsky S, Trudeau M, Hood N, Redwood S: **Adjuvant Treatment and Onset of Menopause Predict Weight Gain After Breast Cancer Diagnosis.** *J Clin Oncol* 1999, **17:**120–129.
10. Irwin ML, Crumley D, McTiernan A, Bernstein L, Baumgartner R, Gilliland FD, Kriska A, Ballard-Barbash R: **Physical activity levels before and after a diagnosis of breast carcinoma.** *Cancer* 2003, **97**(7):1746–1757.
11. van Londen G, Perera S, Vujevich K, Rastogi P, Lembersky B, Brufsky A, Vogel V, Greenspan S: **The impact of an aromatase inhibitor on body composition and gonadal hormone levels in women with breast cancer.** *Breast Cancer Res Treat* 2011, **125**(2):441–446.
12. Francini G, Petrioli R, Montagnani A, Cadirni A, Campagna S, Francini E, Gonnelli S: **Exemestane after tamoxifen as adjuvant hormonal therapy in postmenopausal women with breast cancer: effects on body composition and lipids.** *Br J Cancer* 2006, **95:**153–158.
13. Montagnani A, Gonnelli S, Cadirni A, Caffarelli C, Del Santo K, Pieropan C, Campagna MS, Montomoli M, Petrioli R, Nuti R: **The effects on lipid serum levels of a 2-year adjuvant treatment with exemestane after tamoxifen in postmenopausal women with early breast cancer.** *Eur J Intern Med* 2008, **19:**592–597.
14. Healy LA, Ryan AM, Carroll P, Ennis D, Crowley V, Boyle T, Kennedy MJ, Connolly E, Reynolds JV: **Metabolic Syndrome, Central Obesity and Insulin Resistance are Associated with Adverse Pathological Features in Postmenopausal Breast.** *Clin Oncol* 2010, **22**(4):281–288.
15. Mourtzakis M, Bedbrook M: **Muscle atrophy in cancer: a role for nutrition and exercise.** *Appl Physiol Nutr Metab* 2009, **34**(5):950–956.
16. Kroenke CH, Chen WY, Rosner B, Holmes MD: **Weight, weight gain, and survival after breast cancer diagnosis.** *J Clin Oncol* 2005, **23**(7):1370–1378.

17. Nichols HB, Trentham-Dietz A, Egan KM, Titus-Ernstoff L, HOlmes MD, Bersch AJ, Holick CN, Hampton JM, Stampfer MJ, WIllett WC, Newcomb PA: **Body Mass Index Before and After Breast Cancer Diagnosis: Associations with All-Cause, Breast Cancer, and Cardiovascular Disease Mortality.** *Cancer Epidemiol Biomark Prev* 2009, **18:**1403–1409.
18. Caan BJ, Kwan MI, Hartzell G, Castillo A, Slattery M: **Pre-diagnosis body mass index, post-diagnosis weight change, and prognosis among women with early stage breast cancer.** *Cancer Causes Control* 2008, **19:**1319–1328.
19. Thomson CA, Stopeck AT, Bea JW, Cussler E, Nardi E, Frey G, Thompson PA: **Changes in Body Weight and Metabolic Indexes in Overweight Breast Cancer Survivors Enrolled in a Randomized Trial of Low-Fat vs.** *Reduced Carbohydrate Diets Nutr Cancer* 2010, **62**(9):1142–1152.
20. Jen KLC, Djuric Z, DiLaura NM, Buison A, Redd JN, Maranci V, Hryniuk WM: **Improvement of Metabolism among Obese Breast Cancer Survivors in Differing Weight Loss Regimens [ast] [ast].** *Obesity* 2004, **12**(2):306–312.
21. Irwin ML, Alvarez-Reeves M, Cadmus L, Mierzejewski E, Mayne ST, Yu H, Chung GG, Jones B, Knobf MT, DiPietro L: **Exercise Improves Body Fat, Lean Mass, and Bone Mass in Breast Cancer Survivors.** *Obesity* 2009, **17**(8):1534–1541.
22. Courneya KS, Segal RJ, Mackey JR, Gelmon K, Reid RD, Friedenreich CM, Ladha AB, Proulx C, Vallance JKH, Lane K, Yasui Y, McKenzie DC: **Effects of Aerobic and Resistance Exercise in Breast Cancer Patients Receiving Adjuvant Chemotherapy: A Multicenter Randomized Controlled Trial.** *J Clin Oncol* 2007, **25**(28):4396–4404.
23. Schmitz KH, Ahmed RL, Hannan PJ, Yee D: **Safety and Efficacy of Weight Training in Recent Breast Cancer Survivors to Alter Body Composition, Insulin, and Insulin-Like Growth Factor Axis Proteins.** *Cancer Epidemiol Biomark Prev* 2005, **14**(7):1672–1680.
24. Matthews C, Wilcox S, Hanby C, Der Ananian C, Heiney S, Gebretsadik T, Shintani A: **Evaluation of a 12-week home-based walking intervention for breast cancer survivors.** *Support Care Cancer* 2007, **15**(2):203–211.
25. Knobf MT, Insogna K, DiPietro L, Fennie K, Thompson AS: **An Aerobic Weight-Loaded Pilot Exercise Intervention for Breast Cancer Survivors: Bone Remodeling and Body Composition Outcomes.** *Biol Res Nurs* 2008, **10**(1):34–43.
26. DeNysschen CA, Brown JK, Cho MH, Dodd MJ: **Nutritional Symptom and Body Composition Outcomes of Aerobic Exercise in Women With Breast Cancer.** *Clin Nurs Res* 2011, **20**(1):29–46.
27. Herrero F, San Juan AF, Fleck SJ, Balmer J, Pérez M, Cañete S, Earnest CP, Foster C, Lucía A: **Combined Aerobic and Resistance Training in Breast Cancer Survivors: A Randomized, Controlled Pilot Trial.** *Int J Sports Med* 2006, **27**(7):573–580.
28. Fernández-Lao C, Cantarero-Villanueva I, Ariza-Garcia A, Courtney C, Fernández-de-las-Peñas C, Arroyo-Morales M: **Water versus land-based multimodal exercise program effects on body composition in breast cancer survivors: a controlled clinical trial.** *Support Care Cancer* 2013, **21**(2):521–530.
29. Ibrahim E, Al-Homaidh A: **Physical activity and survival after breast cancer diagnosis: meta-analysis of published studies.** *Med Oncol* 2010, **28**(3):753–765.
30. Irwin ML, McTiernan A, Manson JE, Thomson CA, Sternfeld B, Stefanick ML, Wactawski-Wende J, Craft L, Lane D, Martin LW, Chlebowski R: **Physical Activity and Survival in Postmenopausal Women with Breast Cancer: Results from the Women's Health Initiative.** *Cancer Prev Res* 2011, **4**(4):522–529.
31. Courneya KS, McKenzie DC, Mackey JR, Gelmon K, Reid RD, Friedenreich CM, Ladha AB, Proulx C, Vallance JK, Lane K, Yasui Y, Segal RJ: **Moderators of the effects of exercise training in breast cancer patients receiving chemotherapy.** *Cancer* 2008, **112**(8):1845–1853.
32. Mefferd K, Nichols JF, Pakiz B, Rock CL: **A cognitive behavioral therapy intervention to promote weight loss improves body composition and blood lipid profiles among overweight breast cancer survivors.** *Breast Cancer Res Treat* 2007, **104:**145–152.
33. Demark-Wahnefried W, Case LD, Blackwell K, Marcom PK, Kraus W, Aziz N, Snyder DC, Giguere J, Shaw E: **Results of a Diet/Exercise Feasibility Trial to Prevent Adverse Body Composition Change in Breast Cancer Patients on Adjuvant Chemotherapy.** *Clin Breast Cancer* 2008, **8**(1):70–79.
34. Djuric Z: **A Diet and Exercise Intervention during Chemotherapy for Breast Cancer.** *The Open Obesity J* 2011, **3**(1):87–97.
35. Demark-Wahnefried W, Kenyon AJ, Eberle P, Skye A, Kraus W: **Preventing sarcopenic obesity among breast cancer patients who receive adjuvant chemotherapy: results of a feasibility study.** *Clin Exerc Physiol* 2002, **4**(1):44–49.
36. Irwin ML, Varma K, Alvarez-Reeves M, Cadmus L, Wiley A, Chung GC, DiPietro L, Mayne ST, Yu H: **Randomized Controlled Trial of Aerobic Exercise on Insulin and Insulin-like Growth Factors in Breast Cancer Survivors: The Yale Exercise and Survivorship Study.** *Cancer Epidemiol Biomark Prev* 2009, **18:**306–313.
37. Newman AB, Harris TB, Kupelian V, Visser M, Simonsick E, Goodpaster B, Nevitt M, Kritchevsky SB, Tylavsky FA, Rubin SM, Rubin TB: **Sarcopenia: alternative definitions and associations with lower extremity function.** *J Am Geriatr Soc* 2003, **51**(11):1602–1609.
38. Campbell KL, Foster-Schubert KE, Alfano CM, Wang C-C, Wang C-Y, Duggan CR, Mason C, Imayama I, Kong A, Xiao L, Bain CE, Blackburn GL, Stanczyk FZ, McTiernan A: **Reduced-Calorie Dietary Weight Loss, Exercise, and Sex Hormones in Postmenopausal Women: Randomized Controlled Trial.** *J Clin Oncol* 2012, **30**(19):2314–2326.
39. Breen L, Phillips SM: **Skeletal muscle protein metabolism in the elderly: Interventions to counteract the 'anabolic resistance' of ageing.** *Nutr Metab* 2011, **8**(1):68–68.
40. McDonald CK, Bauer JD, Capra S: **Omega-3 fatty acids and changes in LBM – alone or in synergy for better muscle health? A Review.** *Can J Physiol Pharmacol* 2013, in press.
41. Murphy RA, Mourtzakis M, Mazurak VC: **n-3 polyunsaturated fatty acids: the potential role for supplementation in cancer.** *Curr opin clin nutr metab care* 2012, **15**(3):246.
42. Ries A, Trottenberg P, Elsner F, Stiel S, Haugen D, Kaasa S, Radbruch L: **A systematic review on the role of fish oil for the treatment of cachexia in advanced cancer: an EPCRC cachexia guidelines project.** *Palliat Med* 2012, **26**(4):294–304.
43. Demark-Wahnefried W, Campbell KL, Hayes SC: **Weight management and its role in breast cancer rehabilitation.** *Cancer* 2012, **118**(8 Suppl):2277–2287.
44. Buckley JD, Howe PRC: **Anti-obesity effects of long-chain omega-3 polyunsaturated fatty acids.** *Obes Rev* 2009, **10**(6):648–659.
45. DeFina LF, Marcoux LG, Devers SM, Cleaver JP, Willis BL: **Effects of omega-3 supplementation in combination with diet and exercise on weight loss and body composition.** *Am J Clin Nutr* 2011, **93**(2):455–462.
46. Crochemore ICC, Souza AFP, De Souza ACF, Rosado EL: **ω-3 Polyunsaturated Fatty Acid Supplementation Does Not Influence Body Composition, Insulin Resistance, and Lipemia in Women With Type 2 Diabetes and Obesity.** *Nutr Clin Pract* 2012, **27**(4):553–560.
47. Abete I, Parra D, Crujeiras AB, Goyenechea E, Martinez JA: **Specific insulin sensitivity and leptin responses to a nutritional treatment of obesity via a combination of energy restriction and fatty fish intake.** *J Hum Nutr Diet* 2008, **21**(6):591–600.
48. Hlavaty P, Kunesova M, Gojova M, Tvrzicka E, Vecka M, Roubal P, Hill M, Hlavata K, Kalouskova P, Hainer V, Zak A, Drbohlav J: **Change in Fatty Acid Composition of Serum Lipids in Obese Females After Short-Term Weight-reducing Regimen with the Addition of n-3 Long Chain Polyunsaturated Fatty Acids in Comparison to Controls.** *Physiol Res* 2008, **57**(Supp 1):S57–S65.
49. Krebs JD, Browning LM, McLean NK, Rothwell JL, Mishra GD, Moore CS, Jebb SA: **Additive benefits of long-chain n-3 polyunsaturated fatty acids and weight-loss in the management of cardiovascular disease risk in overweight hyperinsulinaemic women.** *Int J Obes* 2006, **30**(10):1535–1544.
50. Storlien LH, Robertson RM, Hill JO, Reed GW, Kriketos AD, Drougas H, Sharp TA: **Role of weight loss and polyunsaturated fatty acids in improving metabolic fitness in moderately obese, moderately hypertensive subjects.** *J Hypertens* 2001, **19**(10):1745–1754.
51. Thorsdottir I, Tomasson H, Gunnarsdottir I, Gisladottir E, Kiely M, Parra MD, Bandarra NM, Schaafsma G, Martinez JA: **Randomized trial of weight-loss-diets for young adults varying in fish and fish oil content.** *Int J Obes* 2007, **31**(10):1560–1566.
52. Hill A, Buckley JD, Murphy KJ, Howe PRC: **Combining fish-oil supplements with regular aerobic exercise improves body composition and cardiovascular disease risk factors.** *Am J Clin Nutr* 2007, **85:**1267–1274.
53. Noreen EE, Sass MJ, Crowe ML, Pabon VA, Brandauer J, Averill LK: **Effects of supplemental fish oil on resting metabolic rate, body composition, and salivary cortisol in healthy adults.** *J Int Soc Sports Nutr* 2010, **8**(7):31.
54. Munro IE, Garg ML: **Dietary supplementation with n-3 PUFA does not promote weight loss when combined with a very-low-energy diet.** *Br J Nutr* 2012, **108**(8):1466–1474.

55. Rodacki CL, Rodacki AL, Pereira G, Naliwaiko K, Coelho I, Pequito D, Fernandes LC: **Fish-oil supplementation enhances the effects of strength training in elderly women.** *Am J Clin Nutr* 2012, **95**(2):428–436.
56. Smith GI, Atherton P, Reeds DN, Mohammed BS, Rankin D, Rennie DJ, Mittendorfer B: **Dietary omega-3 fatty acid supplementation increases the rate of muscle protein synthesis in older adults: a randomized controlled trial.** *Am J Clin Nutr* 2011, **93:**402–412.
57. Smith GI, Atherton P, Reeds DN, Mohammed BS, Rankin D, Rennie MJ, Mittendorfer B: **Omega-3 polyunsaturated fatty acids augment the muscle protein anabolic response to hyperinsulinaemia-hyperaminoacidaemia in healthy young and middle-aged men and women.** *Clinical sci (London, England: 1979)* 2011, **121**(6):267–278.
58. Boyd M: **Safety of fish oil and omega-3 fatty acids.** *Aust Prescr* 2010, **33**(2):2010.
59. Harris WS: **Expert Opinion: Omega-3 Fatty Acids and Bleeding—Cause for Concern?** *Am J Cardiol* 2007, **99**(6A). Online - http://www.AJConline.org.
60. Winters-Stone K, Dobek J, Nail L, Bennett J, Leo M, Naik A, Schwartz A: **Strength training stops bone loss and builds muscle in postmenopausal breast cancer survivors: a randomized, controlled trial.** *Breast Cancer Res Treat* 2011, **127**(2):447–456.
61. Saarto T, Sievänen H, Kellokumpu-Lehtinen P, Nikander R, Vehmanen L, Huovinen R, Kautiainen H, Järvenpää S, Penttinen H, Utriainen M, Jääskeläinen AS, Elme A, Ruohola J, Palva T, Vertio H, Rautalahti M, Fogelholm M, Luoto R, Blomqvist C: **Effect of supervised and home exercise training on bone mineral density among breast cancer patients. A 12-month randomised controlled trial.** *Osteoporos Int* 2012, **23**(5):1601–1612.
62. Schmitz KH, Ahmed RL, Troxel A, Cheville A, Smith R, Lewis-Grant L, Bryan C, Williams-Smith CT, Greene QP: **Weight Lifting in Women with Breast-Cancer-Related Lymphedema.** *N Engl J Med* 2009, **361**(7):664.
63. Lubans DR, Mundey CM, Lubans NJ, Lonsdale CC: **Pilot randomized controlled trial: elastic-resistance-training and lifestyle-activity intervention for sedentary older adults.** *J Aging Phys Act* 2013, **21**(1):20.
64. Burd NA, West DWD, Staples AW, Atherton PJ, Baker JM, Moore DR, Holwerda AM, Parise G, Rennie MJ, Baker SK, Phillips SM: **Low-Load High Volume Resistance Exercise Stimulates Muscle Protein Synthesis More Than High-Load Low Volume Resistance Exercise in Young Men.** *PLoS ONE* 2010, **5**(8):e12033.
65. Ash S, Reeves M, Bauer J, Dover T, Vivanti A, Leong C, Sullivan TOM, Capra S: **A randomised control trial comparing lifestyle groups, individual counselling and written information in the management of weight and health outcomes over 12 months.** *Int J Obes* 2006, **30**(10):1557–1564.
66. Schmitz KH, Courneya KS, Matthews CE, Demark-Wahnefried W, Galvao DA, Pinto BM, Irwin ML, Wolin KY, Segal RJ, Lucia A, Schneider CM, von Gruenigen VE, Schwartz AL: **American College of Sports Medicine Roundtable on Exercise Guidelines for Cancer Survivors.** *Med Sci Sports & Exercise July* 2010, **42**(7):1409–1426.
67. McCrory MA, Gomez TD, Bernauer EM, Mole PA: **Evaluation of a new air displacement plethysmograph for measuring human body composition.** *Med Sci Sports Exerc* 1995, **27**(12):1686–1691.
68. Levenhagen DK, Borel MJ, Welch DC, Piasecki JH, Piasecki DP, Chen KY, Flakoll PJ: **A Comparison of Air Displacement Plethysmography with Three Other Techniques to Determine Body Fat in Healthy Adults.** *J Parenter Enteral Nutr* 1999, **23**(5):293–299.
69. Minderico CS, Silva AM, Teixeira PJ, Sardinha LB, Hull HR: **A FD: Validity of air-displacement plethysmography in the assessment of body composition changes in a 16-month weight loss program.** *Nutr Metab* 2006, **3:**32–39.
70. Weyers AM, Mazzetti SA, Love DM, Gomez AL, Kraemer WJ, Volek JS: **Comparison of methods for assessing body composition changes during weight loss.** *Med Sci Sports Exerc* 2002, **34**(3):497–502.
71. Plasqui G, Soenen S, Westerterp-Plantenga MS, Westerterp KR: **Measurement of longitudinal changes in body composition during weight loss and maintenance in overweight and obese subjects using air-displacement plethysmography in comparison with the deuterium dilution technique.** *Int J Obes* 2011, **35**(8):1124–1130.
72. Cella D, Tulsky D, Gray G, Sarafian B, Linn E, Bonomi A, Silberman M, Yellen S, Winicour P, Brannon J: **The Functional Assessment of Cancer Therapy scale: Development and validation of the general measure.** *J Clin Oncol* 1993, **11**(3):570–579.
73. Coster S, Poole K, Fallowfield L: **The validation of a quality of life scale to assess the impact of arm morbidity in breast cancer patients post-operatively.** *Breast Cancer Res Treat* 2001, **68**(3):273–282.
74. McNeely ML, Campbell KL, Rowe BH, Klassen TP, Mackey JR, Courneya KS: **Effects of exercise on breast cancer patients and survivors: a systematic review and meta-analysis.** *Can Med Assoc J* 2006, **175**(1):34–41.
75. Lemieux J, Goodwin PJ, Bordeleau LJ, Lauzier S, Théberge V: **Quality-of-Life Measurement in Randomized Clinical Trials in Breast Cancer: An Updated Systematic Review (2001–2009).** *J Natl Cancer Inst* 2011, **103**(3):178–231.
76. Australian Institute of Health and Welfare (AIHW): **The Active Australia Survey: a guide and manual for implementation, analysis and reporting.** In Canberra: AIHW; 2003. Accessed at: http://www.aihw.gov.au/publication-detail/?id=6442467449.
77. Martin GS: *The interviewer-administered, open-ended diet history method for assessing usual dietary intakes in clinical research: relative and criterion validation studies.* Wollongong: University of Wollongong; 2004.
78. American College of Sports Medicine (ed.): *ACSM's Guidelines for Exercise Testing and Prescription 8th Ed.* Philadelphia: Wolters Kluwer/Lippincott Williams & Wilkins; 2010.
79. Innes E: **Handgrip strength testing: A review of the literature.** *Aust Occup Ther J* 1999, **46**(3):120–140.
80. Marfell-Jones M, Olds T, Stewart A, Carter L: *International Standards for Anthropometric Assessment (2007).* 2nd edition. Potchefstroom, South Africa: International Society for the Advancement of Kinanthropometry: School of Biokinetics, Recreation and Sports Science, North West University; 2007.
81. Alexander S, Minton O, Stone PC: **Evaluation of Screening Instruments for Cancer-Related Fatigue Syndrome in Breast Cancer Survivors.** *J Clin Oncol* 2009, **27**(8):1197–1201.
82. Bruce B, Fries J: **The Stanford Health Assessment Questionnaire: a review of its history, issues, progress, and documentation.** *J Rheumatol* 2003, **30:**167–178.
83. Henry N, Giles J, Ang D, Mohan M, Dadabhoy D, Robarge J, Hayden J, Lemler S, Shahverdi K, Powers P, Li L, Flockhart D, Stearns V, Hayes DF, Storniolo AM, Clauw DJ: **Prospective characterization of musculoskeletal symptoms in early stage breast cancer patients treated with aromatase inhibitors.** *Breast Cancer Res Treat* 2008, **111**(2):365–372.
84. Greene JG: **Constructing a standard climacteric scale.** *Maturitas* 1998, **29:**25–31.

doi:10.1186/1471-2407-14-264
Cite this article as: McDonald *et al.*: The muscle mass, omega-3, diet, exercise and lifestyle (MODEL) study – a randomised controlled trial for women who have completed breast cancer treatment. *BMC Cancer* 2014 **14**:264.

3.2.1 Additional information relevant to the primary outcomes

Assessment of Hs-CRP

A full explanation of the analytical processes can be found in Appendix 6. Healthscope pathologies laboratory were used for all results. Coefficient Variations (CV) of the instrument used were documented at 2.3 to 3.7% (see Appendix 6).

In breast cancer populations, an increased risk of cardiovascular disease has been associated with a CRP value of >3.9 (Pierce et al 2008) and risk increased in a dose dependent manner. Considering Hs-CRP is a marker of chronic inflammation, that loss of LBM and higher fat mass is related to an increase in CRP (Dee et al 2010), and its measurement is readily available through routine assessment, it was considered as an appropriate measure. Preliminary evidence also indicates that exercise participation (Fairey et al 2005) and LCn-3 (Tsitouras et al 2008) intake are negatively associated with hs-CRP.

3.3 Ethics Approval

This study proposal was submitted to Uniting*Care* Health Human Research Ethics Committee (HREC) as well as the University of Queensland HREC. This study was granted ethical approval jointly from the Uniting*Care* Health HREC on the 27/10/2010 (Reference #: 1035), and the University of Queensland HREC on the 28/4/2011 (Ref: 2011000079; see **Appendix 1**). Nine variations were applied for and approved by the respective HREC's since initial approval was granted. These variations reflected the need to modify the study design to account for practical issues that arose during the course of the study. The majority of these changes were related to the wording of the participant informed consent document; and to expand the recruitment criteria to the general public, as opposed to referrals only from select consultants in the Wesley Medical Centre. Copies of the final participant information and consent forms (inclusive of the approved variations) are provided in Appendix 3**.**

3.4 Study Design

The study included three groups to compare efficacy of LCn-3 and lifestyle alone or in combination together. An olive oil only group was not included for a number of reasons: 1) The goal of the trial was to determine the efficacy of LCn-3 in comparison to the lifestyle program, compared to a combination of both. A 'no treatment, olive oil only' group was not needed to answer this question; 2) Current evidence indicates that exercise is an important consideration for better outcomes after treatment for breast cancer (Ibrahim and Al-Homaidh 2010, Schmitz et al. 2010), hence inclusion of a 'no treatment' group was not considered ethical. The overall structure of the study including data collection points can be seen in Table 3.1.

TABLE 3.1 OUTLINE AND DESCRIPTION OF INTERVENTION

<table>
<tr><td></td><td colspan="3">Intervention groups</td></tr>
<tr><td>Week number</td><td>N-3</td><td>EP+OO</td><td>EP+N-3</td></tr>
<tr><td>-1</td><td colspan="3">Baseline assessment and data collection</td></tr>
<tr><td>0</td><td colspan="3">Baseline assessment and data collection (cont’d)
Start of capsule consumption – Capsule collection and randomisation</td></tr>
<tr><td>1</td><td rowspan="11">Participants were able to contact the primary investigator at any stage during this time period</td><td colspan="2">General healthy eating and benefits of exercise specific to breast cancer; Resistance training familiarisation</td></tr>
<tr><td>2</td><td colspan="2">Resistance training familiarisation + goal setting for aerobic training; Plant food consumption, practicalities and budget</td></tr>
<tr><td>3</td><td colspan="2">Maintaining a healthy weight/intake – appropriate portions/fats; Resistance training session</td></tr>
<tr><td>4</td><td colspan="2">Mindful eating and hunger; Resistance training circuit</td></tr>
<tr><td>5</td><td colspan="2">Meat, Salt, Supplements; Resistance training</td></tr>
<tr><td>6</td><td colspan="2">Alcohol & Socialising; Resistance training</td></tr>
<tr><td>8</td><td colspan="2">Label Reading; Resistance training</td></tr>
<tr><td>10</td><td colspan="2">Nutrition myths and breast cancer; Resistance training</td></tr>
<tr><td>12</td><td colspan="2">Discussion group regarding keeping the program going; Goal setting for future 12 weeks and beyond</td></tr>
<tr><td>13-14</td><td colspan="3">12 week assessment and data collection & capsule collection</td></tr>
<tr><td>15-24</td><td colspan="3">Ongoing capsule consumption and/or exercise and nutritional habits</td></tr>
<tr><td>25</td><td colspan="3">Post-intervention assessment and data collection
Cessation of capsule provision</td></tr>
</table>

N-3: LCn-3 supplementation only group; Ex+OO: Nutrition and exercise lifestyle program plus olive oil supplementation; Ex+N-3: Nutrition and exercise lifestyle program plus LCn-3 supplementation.

3.4.1 Development of dietary and exercise interventions

The lifestyle program was designed to improve knowledge and practice of physical activity and healthy dietary practices specific to breast cancer. Physical activity and function was addressed through formal range of motion, strength and endurance exercise training. Dietary habits and knowledge was improved through delivery of theory and practical information sessions via powerpoint presentations and facilitated group discussions. A summary of the sessions and is shown in Table 3.1.

All sessions including educational and exercise activities were held in a conference room within the Wesley Research Institute. Parking for the participants were offered at no cost.

Exercise prescription for the Lifestyle Program

A combination of resistance and aerobic exercise training was prescribed in agreement with the ACSM(Schmitz et al. 2010) and ESSA (Hayes et al. 2009) best-practice exercise for cancer survivors position stands. Details of the exercise prescription are shown in Table 3.2**.**

Participants were directed to perform aerobic exercise at home or in the gym to elicit an intensity that related to a rating of 11-14 on the Borg Scale (Appendix 4). Aerobic exercises recommended included but was not restricted to walking, running, cycling, rowing, dancing, gym classes and/or swimming.

Resistance training was performed using a combination of body weight exercises and a GymStick® Figure 3.1. Exercises involved all major muscle groups starting with 4 exercises and building to eight to ten exercises by week eight (progression was prescribed individually during sessions by the primary investigator). Prescribed and resistance progressions can be seen in full in the MODEL Study manual (Appendix 4).

Figure 3.1 Gymstick - resistance training apparatus

Participants were requested to record their activity in a diary that was supplied (Appendix 4). The log was designed to capture daily exercise feedback on intensity, duration and type of training. Due to the resistance of the GymStick® and body weight exercises being considerably lower than that available when using conventional weight machines and free weights, participants were instructed to perform the exercises to temporary fatigue regardless of number of repetitions. Current resistance training guidelines high-load contractions (i.e. ≥70% of 1-RM) for optimal stimulation of muscle growth (American College of Sports 2009), however a recent study in young men has indicated that achieving temporary failure may be more critical for muscle protein synthesis (MPS) than the absolute weight lifted (Burd et al. 2010). The study revealed an equivocal MPS response for four sets of 90% 1-repetition max (1-RM) and 30%1-RM when both were performed to temporary failure. However, a third group that performed 30%1-RM to match the workload of the 90%1-RM (volume of work was the focus rather than temporary failure), experienced significantly lower rates of MPS (Burd et al. 2010). Considering this, exercising to temporary failure was considered a reasonable approach to manage the limitations presented by a semi-supervised intervention that utilised lower tension resistance apparatus. Exercise prescription during the trial is outlined in Figure 3.2.

Exercise, physical activity education and adverse events

Information regarding exercise terminology, decreasing sedentary time and ensuring safe exercise practices were delivered during education sessions. During this time and the supervised exercise sessions, participants were encouraged to ask questions about activity and report any discomforts experienced at home or during supervision.

Nutrition education

The aims of the nutrition education sessions were to improve dietary quality, and inform participants of relevant nutrition topics to prevent misinterpretation of information from media. The content was based on the whole food approach focusing on general healthy eating recommendations suitable for breast cancer survivors including high intake of plant foods, consuming lean meats, low fat dairy, wholegrain breads and cereals, appropriate fat consumption and a reduction of high fat and high sugary foods (Robien, Demark-Wahnefried, and Rock 2011, Pierce et al. 2007, Chlebowski et al. 2006, Harris, Bergkvist, and Wolk 2012, Magee and Rowland 2012). The basic structure and delivery style of the program was adapted from a previous 6-week group healthy eating weight loss program, Fat Booters Incorporated (FBI), published previously (Ash et al. 2006). Notable changes to this program from the original FBI protocol included the exclusion of prescribed energy restriction, and the addition of sessions that covered mindful eating, cancer nutrition myths/media misinterpretation, alcohol and cancer, soy products and nutrient supplementation.

No specific recommendations were given in regards to energy and protein intake. An increase of both has been shown to result in overall body weight and LBM increases of which is known to significantly influence LBM change, and have synergistic effects when combined with exercise training (Norgan and Durnin 1980, Breen and Phillips 2013).

To minimise additional LCn-3 intake, participants were advised to not consume any LCn-3 supplements, nor was there a significant focus on LCn-3 related intake throughout the program.

Nutrition and exercise information for LCn-3 consumption alone

Participants randomised to the N-3 group were advised to live and eat freely. Information available to the public in regards to exercise and nutrition were provided on request. However, all other advice was limited to what they received from their medical team. They were recommended to access publicly available information regarding exercise and nutrition for breast cancer survivors. No nutritional restrictions were placed on this group other than avoiding added consumption of LCn-3 containing supplements.

Freedom to act was an important component of this trial, as part of the research aims to answer if LCn-3 supplementation alone is enough to influence the participants LBM. Currently, there is good

awareness through media and other promotion, that nutrition and physical activity have a role in wellbeing after a diagnosis of breast cancer. Thus, testing if LCn-3 is adequate in influencing outcomes to the same extent as a structured lifestyle program requires those in the LCn-3 only group to live as they would. In this way we determine the necessity for structured education versus the true influences found outside the program.

TABLE 3.2 AEROBIC AND RESISTANCE TRAINING PRESCRIPTION & PROGRESSION

Training/Location	Weeks 1-3	Weeks 4-6	Weeks 7 - 24
Aerobic training Home or Gym Based	2 x 30mins/wk RPE: 11-13	3 x 30mins/wk RPE: 12-14	3-5 x 30-45 mins/wk RPE: 12-14
Resistance training Home based using only the GymStick	2 x 30 min/wk 4 exercises; Repetitions to fatigue 1-2 sets per exercise	2 x 30min/wk 6-8 exercises Repetitions to fatigue 2 sets per exercise	2-3 x 30min/wk 8-10 exercises Repetitions to fatigue 2-3 sets per exercise

RPE: Relative perceived exertion; wk: week;

3.4.2 Omega-3 and Placebo supplementation

Further information about the oil capsules

Placebo capsules consumed by those in Ex+OO contained 1g of light olive oil. The olive oil was treated to remove the majority of its antioxidants during manufacture. The dose of 3g (1.75g EPA and 1.25g DHA per day) of LCn-3 was chosen because LCn-3 absorption into tissue is likely to be maximized at this dose (Dosing was confirmed by Alpha Laboratories, NZ, with documentation provided by the manufacturer on request). Uptake into the tissue is negatively correlated with BMI (Hogg et al. 2006), however findings from our review indicate that in order to achieve maximal LCn-3 uptake into erythrocytes, 1.42g of EPA and 1.12g of DHA may be required (Yee et al. 2010, McDonald, Bauer, and Capra 2013).

Participants were given a 12-week supply of capsules at baseline and 12weeks. It was requested that all bottles and unconsumed pills were returned to the primary investigator. Blackmores Pty Ltd supplied all capsules for the trial *gratus.* They were approached for provision of the capsules as they already had a high strength 1000mg omega-3 product that achieved appropriate dosing. The Blackmores' liaison was only involved for the provision of capsules and generation of the randomised sequence.

3Blinding of capsules

The N-3 groups capsules were open-label, while the Ex+OO and Ex+N-3 groups' capsules were blinded to participants and the research team. The Blackmore's representative who mediated the production & delivery of the capsules also performed the blinding sequence. The blind was not broken until all participants had completed the 24 week protocol and all data was entered and cleaned.

3.4.4 Further information on Recruitment

The period of recruitment took place between July 2011 and January 2013. Original projections of recruitment were an uptake of 8-12 participants per month for 14 months, however, due to this recruitment being significantly lower than expected, the recruitment window was expanded. The primary method for recruitment was via Brisbane based oncologists (medical, radiological and surgical) at the Wesley Hospital and the Mater Hospital. In addition, recruitment materials (Appendix 2) and presentations were given at breast care centres, outpatient chemotherapy units, local radio, via social media, local newspapers, national and local television news and relevant email lists. Individuals were asked to contact the primary investigator and a preliminary screening was carried out. If they met the criteria for the study they were sent a Participant Information and Informed Consent Form (Appendix 3) to read and further consider the study. If they wished to participate, they were informed of the next assessment day, allocated a time convenient to them, and on signing the consent form were eligible to begin the baseline assessments.

Eligibility Screening

Upon calling or emailing to express interest in participation, individuals were asked a series of questions relating to the eligibility criteria (Appendix 3). If they met the criteria they were offered a choice of times on the next assessment day. As eligibility was in part determined by body composition, final eligibility was determined as a result of weight and height measures (BMI) taken at the time of the baseline assessment.

Women who had completed treatment for breast cancer within the last 12 months were chosen for the study as it has been shown that the rate of body composition change is greatest for the six to 12 months following treatment (Makari-Judson et al 2007). Therefore, if the intervention had an effect it was expected that differences between groups would be more pronounced. Both pre- and postmenopausal women were included in our study, as a review of the studies to date have reported a general lack of premenopausal participation in lifestyle interventions. In addition, both populations were included to increase the potential pool of recruitment, and so that the effect of the intervention could be assessed in both populations. Women were excluded if their BMI was less than 20kg/m^2 or greater than 35kg/m^2. It has been noted that women who experience significant

loss of weight are at a greater risk of mortality (Nichols et al 2009, Caan et al 2008). The research team determined from clinical experience that women who were underweight would require a more specialised dietary and exercise intervention to prevent further weight loss following treatment. For those with a BMI of greater than 35, it was determined a more specialised dietary intervention would be required. In addition, exercise physiology considerations differ for this population. It was determined a more individualised and intensive program was necessary to allow for individual disease and biomechanical risk. Those with a chronic disease such as diabetes and cardiovascular disease were excluded as best practice for these individuals is an individualised program to address their specific condition; as information relating to those diseases, effects of medications and contraindications to exercise were not covered in the program.

3.4.5 Secondary Outcome Measures

The following section contains the considerations for the selection of and the procedure for the secondary outcome measures chosen. It should be noted to the reader that if the methodology was comprehensively accounted for in the Methods Paper – Section 3.2, then they are not accounted for in the following text.

Measures of adherence to the intervention

Physical activity during the 12 week intervention

Both groups who completed the lifestyle program (Ex+N-3 and Ex+OO) were asked to report their weekly exercise throughout the first 12 weeks. The information gathered included: the number of times resistance and aerobic exercise was performed, for resistance exercise, sets, reps and specific exercises were recorded. For aerobic, time and intensity (as rated on the Borg Scale) was recorded. Changes in activity levels were also accounted for in the Active Australia Survey and through use of the accelerometer.

Physical activity during follow up: weeks 13-24

All participants were asked to record their level of physical activity after the 12wk assessment in a diary provided. Information collected included the same data as through the first 12 weeks.

Waist girth

Waist girth was measured as a marker of abdominal obesity and cardio-metabolic health, both of which are relevant to co-morbidities in general and breast cancer survivor populations (Healy et al. 2010, Thomson et al. 2009). As change in body composition over time was of relevance to this study, the site defined in the International Standards for Anthropometric Assessment(Marfell-Jones et al. 2007) was used. The metal tape measure was positioned at *'the narrowest point between the*

lower coastal (10th rib) border and top of the iliac crest, perpendicular to the long axis of the trunk' on the skin (i.e. sports bra or swimwear). The participant was asked to relax and to exhale during measurement. The tape measure was snug, but not compressing the skin.

Hip girth

Hip girth was measured as an additional marker of fat tissue change. Current evidence does not indicate that there is an increased risk of co-morbidity associated with fat deposition around the gluteal, however it is an area that is sensitive to body composition change (Rocha et al. 2008). The measure was taken after participants were asked to stand in bike shorts or swim wear with heels together, and relaxed gluteal muscles. The tape was placed around the buttocks '*at the level of their greatest posterior protuberance, perpendicular to the long axis of the body'* (Marfell-Jones et al. 2007).

Fatigue

Fatigue is a common side effect after treatment for breast cancer (Meeske 2007). Fatigue was measured using the FACT-F tool, a stand-alone 13-item scale that has been used in a number of studies assessing cancer related fatigue. A validation study carried out in cancer populations (24% of whom had breast cancer) indicated a clinically important difference was a change in score of 3.0 (Cella et al 2002). In addition, physical fitness and activity has been shown to be negatively associated with perceived fatigue (McNeely et al. 2006). One controlled exercise trial in breast cancer survivors indicated statistically improved fatigue as measured by FACT-F (Courneya, Mackey, et al. 2003) which is calculated through the addition of the FACT-G to the fatigue specific 13-item survey. Each item can be answered on a five-point scale. Scores can range between 0 and 52 with lower scores indicating greater fatigue. When assessing cancer-related fatigue syndrome (CRFS), compared to the recognised standard or diagnostic interview and structured psychiatric interview, the FACT-F scale was reported to have a sensitivity of 80%, specificity of 71%, a positive predictive value of 55% and negative predictive value of 89% (Alexander, Minton, and Stone 2009).

Dietary history using the Diet History Questionnaire

The primary investigator (an accredited practicing dietitian) carried out a semi-structured diet history of the participants overall food intake using the Diet History Questionnaire (Smart Foods Centre, University of Wollongong – Appendix 3). On day 7 of the assessment period, participants were asked to complete the form to the best of their ability, at which point the clinician clarified portion sizes and helped the participant complete items left blank. This particular tool has been validated for overall energy intake and total LCn-3 intake (Martin 2004). The diet history takes

amount and frequency of consumption into consideration for average daily intake (kilojoules) over the previous month. Nutrient intake was analysed using Foodworks 7, Xyris Software.

Objective physical activity

Increased time spent in sedentary activity is related to increased risk of heart disease and diabetes in general populations (Dunstan et al. 2010), and breast cancer survivors are at heightened risk of these diseases following treatment (Demark-Wahnefried, Campbell, and Hayes 2012). Objective physical activity was measured using a uniaxial accelerometer (GT1-M, Actigraph, USA). Participants were asked to wear the accelerometer for all waking hours between Day one and Day seven of each assessment period. Participants were instructed to attach the accelerometer to their hip (belt line) and asked to maintain its consistency of placement for all wear-time. At least four full days (10 hours) of data was required for the data to be classified as valid (Dunstan et al 2010). Accelerometers calculate movement in counts/minute. Sedentary activity is measured as <100 counts/min; light activity as 100-1951 counts/min; moderate activity as 1952-5724 counts/min; and vigorous activity >5724 counts/min. Excessive scores of >20,000 counts/min will be excluded. Non-wear time was defined as intervals of at least 60 consecutive minutes of zero counts, with allowance for up to 2 min of observations of less than 50 counts/min within the non-wear interval. These measures have been used to ensure homogeneity with other studies, and validation studies using the same apparatus (Dunstan et al. 2010).

Self-reported Physical Activity

Self reported physical activity was measured by the Active Australia questionnaire. The tool collects reported time spent in light, moderate and vigorous physical activity, it is a reliable and validated tool against accelerometry, and for assessing changes in daily physical activity as short as 13 days apart (Brown et al. 2008).

Physical fitness and muscle function tests

All assessments were performed under the supervision of the primary investigator, an accredited exercise physiologist. A standardised script was used to maintain consistency in verbal instructions and encouragement.

Sub-maximal exercise

Higher aerobic fitness is strongly associated with lower risk of cardiovascular disease, diabetes, cancer related fatigue and improved overall mortality. Aerobic fitness was measured using the modified Balke protocol on a treadmill in the cardiovascular rehabilitation unit of the Wesley Hospital. Participants were asked to abstain from caffeinated beverages, vigorous exercise for 2 days before the test, and continue all normal medications on the day of the test. Pre-test measures of

blood pressure and heart were taken to ensure the participant was safe to complete exercise. Participants with high blood pressure (>180/110) were not allowed to complete the test, as per best practice guidelines (Sharman and Stowasser 2009) and referred to their oncologist for further cardiovascular screening. The modified Balke protocol is a graded exercise test that consists of 7 stages, each of which is 3 minutes in duration (see Appendix 3). The protocol was designed to increase in intensity until a heart rate equal to 85% of estimated heart rate max (or 70% of heart rate reserve) was reached. The test was terminated when this heart rate was achieved, or when the participant chose to stop, whichever came first. In case of cardiac or injurious event, a 'Code' was to be called and the participant was to be referred to The Wesley Hospital Emergency Department. Safety of this test has been confirmed through numerous studies using the same population and is a recognised assessment for estimated VO_{2max} (Jones et al. 2008).

LBM function tests

Upper and lower body muscular strength-endurance and handgrip strength were measured using the 1-minute push up, 1-minute sit-to-stand and a handgrip dynamometer, respectively. These were included to measure the improvement elicited from any resistance training performed during the study period. The tests followed a standard protocol outlined in the ACSM's Guidelines for Exercise Testing and Prescription (8^{th} Edition) (American College of Sports Medicine 2010).

The 1-minute push-up test involved the participants performing as many consecutive push-ups (knees on ground) as possible in one minute. Technique was monitored to ensure consistent measurement and to minimize risk of injury. Safety of this test has been demonstrated in other populations (Suni et al. 1998), while more advanced tests, e.g. bench press 1-RM has been performed in numerous breast cancer interventions with similar (Schmitz, Ahmed, et al. 2005), and more 'at-risk' populations (Schmitz et al. 2009, Courneya et al. 2007). Participants experiencing lymphoedema were asked to wear their custom garment during the exercise and given specific arm mobility exercises before permitted to complete the test. Participants with upper limb injuries/limitations were allowed to decline the test.

The one-minute squat test involved the participant performing as many sit-stand movements from a chair (43cm height) as they could in one-minute (American College of Sports Medicine 2010). The count included full sit-stand movements only, and participants were encouraged to go as fast as they could. Lower body injuries precluded participants from completing this test. This type of assessment has been used safely in breast cancer interventions previously (Herrero et al. 2006).

Handgrip strength was measured using a handgrip strength dynamometer (Baseline Smedley Spring). Participants were instructed to use their dominant hand, maintain their shoulder in an adducted and neutrally rotated position, with elbow flexed at 90 degrees, forearm in a neutral position, and the wrist between 0 and 30 degrees extension and between 0 and 15 degrees ulnar

deviation (Innes 1999). Handgrip strength was chosen as it provides an indication of overall muscle strength, and has been shown to improve in previously studies cancer populations as a result of anti-inflammatory supplementation (Cerchietti, Naviganteac, and Castroa 2007).

Lymphoedema index using Bioelectrical Impedance Spectroscopy (BIS)

Lymphoedema index (L-Dex) is a measure of the extra-cellular fluid (ECF) and subclinical changes of ECF of the upper limbs using a low frequency bio-impedance machine (Impedimed XC Scanner). Electrodes were placed at each end of the participants arm as per the Impedimed protocol. This device uses an 'impedance ratio' to assess unilateral lymphoedema of the arm. It has been shown to detect subclinical lymphoedema in breast cancer survivors, and served as an added safety measure for potential lymphoedema exacerbations during the program. Typically, lymphoedema is indicated if an individuals score is $\geq$10, or if intra-participant measures change >3 standard deviations between assessments, i.e. a score increase of $\geq$10; thus, if a participant records a negative number, e.g. -2.5 at baseline, a subsequent score of +7.5 (+10 difference) would indicate lymphoedema. A recent review indicated that BIS is as effective and potentially more clinically relevant than arm water displacement, perometry and arm girth for management of lymphoedema (Ward 2009).

Measure of pain and function using the Health Assessment Questionnaire-Disease Index (HAQ-DI)

Breast cancer survivors are at increased risk of arthralgias after treatment. Incidence is higher in those who received hormonal therapy, and higher still in those who received aromatase inhibitors (Din et al. 2010). The HAQ-DI is composed of 20 items in 8 categories (Dressing and Grooming, Hygiene, Arising, Reach, Eating, Grip, Walking, Activities of Daily Living (ADL). Items were developed to be mutually exclusive and collectively exhaustive of activities related to physical functioning. Each category has at least two sub-category questions. Within each category, patients report the amount of difficulty they have in performing the specific sub-category items. There are four response options ranging from 'No Difficulty' to 'Unable to Do', scored 0-3. A recent review of measures to assess quality of life in relation to physical function and joint pain as a result of AIs has suggested that the HAQ-DI is currently a preferred tool (Din et al. 2010). However, specific validation within breast cancer populations have not been performed, which is mainly due to the lack of 'Gold Standard' assessment of AI related arthralgias. The HAQ-DI was originally validated for measurement of change in rheumatoid arthritis (Cardiel et al 1993).

Measure of menopausal symptoms using the Greene Climacteric Scale (GCS)

Menopausal symptoms are a frequent and troublesome side effect of breast cancer therapy in women of all ages. Hot flashes, night sweats, sexual dysfunction, poor sleep and tiredness

frequently occur following breast cancer treatment (Hickey et al. 2008). The GCS is a 21-item questionnaire that measures a variety of menopausal symptoms on a 4-point Likert scale (0 = “not at all” to 3 = “extremely). Three separate sub-scales measure vasomotor symptoms, somatic symptoms, psychological symptoms, and an additional probe related to sexual function. Psychological symptoms can be further sub-divided to measure anxiety and depression. The Scale has also been used to identify menopausal women who are severely and possibly clinically anxious and/or depressed (Greene 1998).

TABLE 3.3 JUSTIFICATION AND ACCURACY OF OUTCOME MEASURES

Inclusion of Hs-CRP as a marker of inflammation	*Justification* - Increased levels of CRP have been associated with all cause mortality in breast cancer populations (Pierce et al 2008). - Hs-CRP is a general marker for low-grade chronic inflammation and is commonly used in general practice (Libby et al 2002). - Previous studies indicate that inflammation is thought to drive LBM loss (Fearon et al 2006) and be related to fatigue in cancer survivors *Accuracy* - All measures were performed by the same laboratory group – Healthscope. - All blood was drawn in a fasting state, at the same time of day - Acute illness within the previous 5 days was a reason for exclusion or test delay.
Inclusion of the FACT- B+4 tool for quality of life	*Justification* - The FACT-B (+4) tool is one of the two most commonly used tools for assessing quality of life in populations with breast cancer (Lemieux et al 2011). - Change in FACT-B score has been concurrently reported with improvements in cardiorespiratory fitness (McNeely et al 2006). *Accuracy* - Questionnaires were delivered before body composition results to reduce the effect of results to bias perceived wellness. - The official FACT-B+4 was delivered to ensure consistency with other studies (Cella et al 1997).
Waist & Hip Girth Measurement	*Justification* - Waist-to-hip and Waist circumference are associated with risk of breast cancer (Harvie et al 2003, Connolly et al 2002). - Waist circumference is associated with metabolic syndrome in women who have completed breast cancer (Healy et al 2010). - It provides information on distribution of fat (abdominal fat mass), whereas the BODPOD describes only total fat volume. - Abdominal obesity is related to markers of inflammation (Festa et al 2001), thus measurement allows observation of this relationship. This is relevant to the effects of LCn-3 (Tsitouras et al 2008) and exercise (Fairey et al 2005) in relation to CRP.

	Accuracy - The research assistant was trained and tested for inter- rater reliability with an Accredited Level 1 ISAK anthropometrist. - Measure of waist and hip were taken in the same type of clothing (lycra swimmers) - Measurement was made in accordance with ISAK protocols for waist and hip girths
Interviewer assisted Diet History Questionnaire (DHQ)	*Justification* - Open ended diet history method allows detailed collection of portion size and variations in intake (Martin et al 2004). Which is likely to improve accuracy of energy intake. - As opposed to a multiple-pass 24 hour recall method, the DHQ had a lower time burden as it was conducted at the same time other testing, ensuring higher completion. - Accurate assessment of energy and protein intake is important for controlling dietary intake when assessing body composition change. *Accuracy* - The DHQ was conducted using the methodology outlined in the original validation study that indicates good sensitivity to change in dietary intake (Martin 2004) - The same Accredited Practising Dietitian conducted every assessment at each time point - Known volumes and quantities were used in the same fashion for all participants
Objective Physical Activity 7-day uniaxial accelerometry	*Justification* - Objective measurement of physical activity through accelerometry is positively associated with cardio-metabolic morbidity, inflammation (Healy et al 2011) and may be related to body composition changes after breast cancer treatment (Lynch et al 2009) - Sedentary time has been related to physiological changes that may be related to an increase in inflammation and subsequent LBM loss (Hamilton et al 2007) *Accuracy* - Participants maintained a written log of 'wear time' that was cross-referenced at each time point. - Participants wore the unit on the same anatomical location at each

	measurement point - Participants that did not wear the unit for a minimum of 10 hours for 4 days were not included in the data - Activities like water-sports were accounted for via the 7-day physical activity record kept as part of their physical activity journal, and through the modified Active Australia Survey.
Lymphoedema-index using (BIS)	*Justification* - Lymphoedema is an important clinical consideration for women treated for breast cancer, who have had lymph glands removed (DiSipio et al 2013). - Sub-clinical assessment of lymphedema is able to be conducted using BIS (Rockson et al 2007). *Accuracy* - All assessments were carried out by the same trained research assistant with experience in measurement of BIS. - Procedures closely followed manufacturer and clinical guidelines

Muscle function tests: 1-min Push up; 1 min Squat test; Hand grip strength; Sub-maximal VO_2 treadmill test	*Justification* - Increased muscle strength are beneficial to overall health, quality of life and mortality (Newman et al 2006, Ruiz et al 2008). These changes can occur without changes in body composition (Schmitz et al 2009). Thus their measurement gives a greater insight into physiological response to the intervention. - Exercise tests are another tool to validate the adherence to the strength training prescribed; upper body and lower body strength training was a focus of the program. - Muscle strength is associated with LBM size, thus controlling for the change in LBM due to strength increases may allow a clearer observation of the effect of LCn-3 on LBM - Improvements in cardiorespiratory fitness are related to a improve quality of life in a breast cancer population (McNeely et al 2006). Participation in aerobic training designed to increase cardiorespiratory fitness has been shown to improve body composition (Irwin et al 2009) - Grip strength has been shown to correlate positively with LCn-3 intake (Robinson et al 2008) *Accuracy* - The same accredited exercise physiologist conducted all assessments at all time points. - Tests were conducted in accordance with the American College of Sports Medicines Official Guidelines for Clinical Exercise Testing (ACSM 2010). - Tests were conducted in the same order at each time point. I.e. Day 1: Grip strength and then treadmill; Day 7: Push ups and then squats. This was to ensure that fatigue from a previous test was not a factor in the results. - Proper technique was demonstrated and monitored at each time point. - Time to reach 85% of estimated max heart rate was recorded for the treadmill test. Many of the participants did not have three readings at >115bpm as per description in the protocol. This allowed generation of a continuous data point. This continuous data point was statistically similar to the stage reached (i.e. counting up in units of 1-minute). Thus stage completed was used for analysis. - Grip strength was calculated as a maximal result over 3 tests on both hands, with posture and movement monitored at each time point.

Hs-CRP: High sensitivity- C-reactive protein, LCn-3: Long chain omega-3 fatty acids; FACT-B: Funcational Assessment during Cancer Therapy – Breast; ISAK: International Society for the Advancement of Kinanthropometry; LBM: Lean body mass; DHQ: Diet History Questionnaire; bpm: Beats per minute; BIS: Bio-Impedance Spectroscopy.

3.5 Data Analysis

3.5.1 Analysis of baseline for cross-sectional study

Baseline characteristics were compared between treatment types and stages of disease using independent samples t tests or ANOVA for normal data, and Mann Whitney-U tests or Kruskal Wallis for non-normal data, respectively. Spearman's correlation coefficient was used to assess the strength of bivariate associations. Percentage (%) of time spent in moderate and vigorous activity were grouped together into one variable: '% time in ≥ moderate activity'. To assess the significance of age- and/or weight-adjusted associations between an outcome and a potential predictor, multivariable linear regression was used. Multivariable linear regression was used to model LBM as a function of various markers of fitness while also controlling for total body mass. Only those with full data sets were included in the models. The variables considered for inclusion in the model were those that were individually associated with LBM after adjusting for age and weight. Markers of fitness were added to the model sequentially, with the order determined by decreasing r-values. A predictor was only retained in the model if its coefficient was significantly different from zero at the 0.05 level. Adjusted R-squared was used to compare nested models. Models were also fitted that included interaction terms that explored the respective LCn-3 indices combined with fitness markers on LBM.

3.5.2 Analysis of baseline post randomisation

Baseline demographic and disease related characteristics were summarised by group as count and percent for categorical variables and number, mean and standard deviation for continuous variables. To compare the three treatment groups at baseline, a chi square test or Fishers exact test was used for categorical variables and a one-way analysis of variance (ANOVA) or Wilcoxon rank-sum test for continuous variables. Baseline demographic and disease-specific characteristics that differed among groups were considered for covariate adjustments in analysis of all outcomes. Measurements collected longitudinally will be summarised by group as number, mean and standard deviation, or standard error (baseline, 12wk, 24wk). Absolute change from baseline will be calculated by subtracting the baseline measurement from the 12 week and 24 week measurements; percent change from baseline will be calculated by dividing the 12-week and 24-week measurements by the baseline measurement. Measurements include: body composition, quality of life, C-Reactive Protein, physical activity and sedentary time, aerobic fitness, muscular endurance, muscle strength, dietary intake, waist and hip girth, joint pain and physical function and menopausal symptoms. All outcome data will be visually inspected for normality. In the case of the data being not normal non-parametric analyses will be used.

3.5.2 Analysis of intervention data

Differences in demographic data between groups were assessed by ANOVA and Kruskal Wallis tests, for parametric and non-parametric data, respectively. The Friedman test and repeated measures ANOVA were used to determine within group differences over 3 time points, and Wilcoxon Signed Rank tests and paired t-tests with Bonferroni adjustment for multiple comparisons were used to determine change within-groups between two time points. The effects of treatment on the dependent measures were analysed by a 3 x 3 factorial repeated measures ANOVA with group treatment (N-3 vs EP+OO vs EP+N-3), exercise treatment (EP+OO & EP+N-3 or N-3), LCn-3 treatment (N-3 & EP+N-3 or EP+OO) and synergy treatment (EP+N-3 or EP+OO & N-3) at three time points. Intention-to-treat was used for all analyses. Contrasts were constructed to compare pair-wise differences among the three treatment groups at each time point. Similar mixed models will be fit to evaluate the effect of adjusting for covariates. In addition to the fixed effects for time, treatment, time by treatment and baseline value, covariates at baseline identified as statistically different among the three groups and covariates known or hypothesised to be associated with the particular change from baseline outcome will be evaluated as fixed effects.

Chapter 4 – Findings from the baseline cross-sectional study

This chapter is divided into 2 sections: Section 1 is a published manuscript that reports overall findings from the baseline assessment before randomisation. This paper also explores cross sectional associations between LBM, demographical variables and lifestyle factors that were measured. Section 2 includes additional analyses that were not included in the published manuscript, but were relevant to our theoretical model.

The major findings from the baseline cross-sectional analyses add new information to the observational evidence base for women who have completed breast cancer treatment. Measures of physical activity and LBM function were significantly positively associated with LBM after adjusting for weight and age. In addition, a threshold effect for physical function and LBM was present. With further exploration, recommendations for exercise volume and optimal body composition could be created to give more specific guidance to women who have been treated for breast cancer.

Considering that previous studies have only indicated non-modifiable or treatment related factors to be associated with LBM change, our observations provide the first evidence for modifiable lifestyle factors to play an important role. This data agrees with intervention data from intervention trials that have noted improvements in LBM after exercise training.

4.1 Published manuscript #4

Muscle function and omega-3 fatty acids in the prediction of lean body mass after breast cancer treatment. Within this paper an abbreviated table of demographic information appears. This detail is covered comprehensively between Table M5-1 in published manuscript #5 – Section 5.1.1, with additional data reported in Table 5.2.

SpringerPlus
a SpringerOpen Journal

RESEARCH Open Access

Muscle function and omega-3 fatty acids in the prediction of lean body mass after breast cancer treatment

Cameron McDonald*, Judy Bauer, Sandra Capra and Mary Waterhouse

Abstract

Background: Decreased lean body mass (LBM) is common in breast cancer survivors yet currently there is a lack of information regarding the determinants of LBM after treatment, in particular, the effect of physical activity and dietary factors, such as long-chain omega-3 fatty acids (LCn-3) on LBM and LBM function. This cross-sectional study explored associations of LBM and function with LCn-3 intake, dietary intake, inflammation, quality of life (QOL) and physical fitness in breast cancer survivors to improve clinical considerations when addressing body composition change.

Methods: Forty-nine women who had completed treatment (surgery, radiation and/or chemotherapy) were assessed for body composition (BODPOD), LCn-3 content of erythrocytes, C-reactive protein (CRP), QOL, dietary intake, objective physical activity, 1-min push-ups, 1-min sit-stand, sub-maximal treadmill (TM) test, and handgrip strength.

Results: After adjustment for age, LBM was associated with push-ups ($r = 0.343$, $p = 0.000$), stage reached on treadmill (StageTM) ($r = 0.302$, 0.001), % time spent ≥ moderate activity (Mod + Vig) ($r = 0.228$, $p = 0.024$). No associations were seen between anthropometric values and any treatment, diagnostic and demographical variables. Body mass, push-ups and StageTM accounted for 76.4% of the variability in LBM (adjusted r-square: 0.764, $p = 0.000$). After adjustment docosahexanoic acid (DHA) was positively associated with push-ups ($\beta=0.399$, $p = 0.001$), eicosapentanoic acid (EPA) was negatively associated with squats ($r = -0.268$, $p = 0.041$), with no other significant interactions found between LCn-3 and physical activity for LBM or LBM function.

Conclusion: This is the first investigation to report that a higher weight adjusted LBM is associated with higher estimated aerobic fitness and ability to perform push-ups in breast cancer survivors. Potential LCn-3 and physical activity interactions on LBM require further exploration.

Keywords: Breast cancer; Omega-3 fatty acids; Lean body mass; Fitness; Nutrition; Exercise

Introduction

Loss of lean body mass (LBM) and simultaneous gains in fat mass are amongst the most common side effects following treatment for breast cancer (Mcdonald et al. 2011). This pattern of body composition change is distressing for the survivors and it is related to higher levels of chronic inflammation (Mourtzakis & Bedbrook 2009), and a greater risk for metabolic syndrome (Healy et al. 2010) and its related diseases (Healy et al. 2010; Pierce et al. 2009). A growing literature has established LBM, and in particular skeletal muscle tissue, as an influential organ in hormonal, immune and metabolic function (Pedersen & Febbraio 2012). Lifestyle factors such as physical activity and nutrient intake can enhance LBM size (Irwin et al. 2009) and function, (Courneya et al. 2007; Schmitz et al. 2005) and have also been associated with improved survival (Ibrahim & Al-Homaidh 2010) and quality of life (Mcneely et al. 2006) after treatment for breast cancer. Taken together, LBM is becoming an important marker for women who have been diagnosed with breast cancer.

* Correspondence: c.mcdonald4@uq.edu.au
University of Queensland, Brisbane 4059, Australia

Findings from observational studies have indicated that chemotherapy has been associated with declines of LBM during and after treatment (Cheney et al. 1994; Demark-Wahnefried et al. 1997; Demark-Wahnefried et al. 2001; Gordon et al. 2011; Kutynec et al. 1999), however not all trials have reported LBM loss after chemotherapy (Campbell et al. 2007). In contrast, associations between higher LBM and aromatase inhibitor hormonal therapy have been reported in three different data sets (Francini et al. 2006; Montagnani et al. 2008; Van Londen et al. 2011). Modifiable variables such as dietary intake and physical activity have not been extensively explored with regard to LBM change in breast cancer populations. Some evidence exists for an association between decreased physical activity and increased adiposity (Irwin et al. 2005), while mixed results have been reported in relation to dietary intake and adiposity, (Sheean et al. 2012) however a deeper understanding of physical activity, dietary factors and LBM change are needed to better guide clinicians in the post-treatment period.

Long chain omega-3 fatty acids (LCn-3) are established as anti-inflammatory agents and have been shown to protect LBM in cancer populations (Dewey et al. 2001; Murphy et al. 2012; Ries et al. 2012; Van Der Meij et al. 2011). However, conclusions from reviews of intervention studies in cancer populations investigating the effect of LCn-3's on LBM have been mixed (Murphy et al. 2012; Ries et al. 2012). Typically, older studies have shown a protective effect for LBM when the appropriate dose of LCn-3 is consumed (Fearon et al. 2006; Fearon et al. 2003). More recent studies investigating 2 g of EPA LCn-3 supplementation in individuals undergoing chemotherapy for non-small cell lung cancer (NSCLC) have shown significantly greater attenuation of LBM and improved levels of intra-muscular triglyceride (IMTG), compared to those not supplementing. (Murphy et al. 2010; Murphy et al. 2011). In non-cancer populations the effect of LCn-3 on LBM has been minimal, with the majority of controlled trials indicating limited clinical effect (Mcdonald et al. 2013b).

Recent research has indicated that a greater effect may be seen when LCn-3 s are combined with an anabolic stimulus (Mcdonald et al. 2013b; Rodacki et al. 2012; Smith et al. 2011a; Smith et al. 2011b). Three small, well controlled studies combined LCn-3 supplementation with exposure to an anabolic stimulus, i.e. hyperinsulinaemic/hyperaminoacidaemic clamp or resistance training. Two reported an increased muscle protein synthetic (MPS) response to for young healthy (Smith et al. 2011b), and elderly participants (Smith et al. 2011a), yet LCn-3 alone made no difference to basal MPS. The third study that used resistance training reported increased peak torque development for the supplemented group above that of the group who received the resistance training program only (Rodacki et al. 2012). Considering LBM function, measured by strength or power development, may be more important to health outcomes than absolute values of LBM, (Newman et al. 2006; Ruiz et al. 2008) further investigations are required.

Therefore, the objectives of this cross-sectional study was to explore associations of LBM and LBM function in the context of LCn-3 intake, dietary energy and protein intake, inflammation, quality of life (QOL) and parameters of physical fitness and activity in women who had completed breast cancer treatment. A secondary goal was to determine the effect of interactions between tissue content of LCn-3 and markers of physical fitness on LBM after treatment for breast cancer.

Methods

Study design

All participants provided written informed consent. The data presented here was collected as the baseline assessment for a 6-month 3-arm randomized controlled trial (RCT) investigating LBM in women who have completed treatment for breast cancer. Detailed rationale study protocol for the full trial has been published previously (Mcdonald et al. 2013a). The study was approved by the Uniting Care (UCH HREC: #1034) and the University of Queensland (#2011000079).

Participants

Participants were invited to participate through hospital breast cancer oncology centres, radio advertising, social media and breast cancer research registries in Brisbane, Australia. Baseline assessment occurred over one week, which included two visits 7 days apart.

Eligibility

Women ≥18 years of age; had been diagnosed with early stage breast cancer (Stage 0-IIIa as determined by the American Joint Committee on Cancer Care); had successfully completed surgery, radiation and/or chemotherapy in the last 12 months (participants could be currently receiving endocrine and/or herceptin therapy); were able to perform moderate intensity physical activity, and have a BMI of >20 and <35 kg/m^2 were eligible for enrolment. Participants were excluded if they had presence of metastatic growth or local/distal recurrence of cancer; a diagnosis of cardiovascular disease or diabetes; or, consumed >1 g of eicosapentanoic acid (EPA) and docosahexanoic acid (DHA) LCn-3 s combined per day.

Measures

Anthropometric variables

Height was measured to the nearest 0.5 cm using a stadiometer (Seca). Weight to the nearest 0.1 kg, LBM and

fat mass were measured using the BODPOD digital scales and air displacement plethysmography (ADP) pod (BODPOD, COSMED USA Inc), respectively. Before each assessment day, the BODPOD scales and air chamber were calibrated as per the manufacturer's instructions using known weights and volumes. All measures were performed by a certified BODPOD assessor. Results were expressed as percentage LBM and body fat of total weight, then absolute LBM was calculated giving a value in kilograms of LBM.

Quality of Life (QOL)

QOL was measured using the Functional Assessment of Cancer Therapy- Breast + 4 (FACT-B + 4) tool (Cella et al. 1993). That FACT-F subset of questions was also added to capture participant fatigue. Higher scores are representative of better well-being.

Diet history

Dietary intake was measured by the practitioner assisted Diet History Questionnaire (Martin 2004). Participants were asked to complete the questionnaire based on their intake over the last month. An accredited practicing dietitian reviewed the questionnaire with the participant to clarify portion sizes and other relevant details. Nutrient analysis was carried out using Foodworks 7 (Xyris Software).

Blood analyses

Fasting high sensitivity-C Reactive Protein (CRP) was measured using a latex-enhanced immunoturbidimetric assay of blood serum. The 8.5 ml sample of whole blood was collected and analysed for CRP, then frozen at −20°C for transport to Victoria, Australia for fatty acid testing.

Lipids from red cells were extracted with chloroform methanol mixture. The fatty acids were trans-esterificated to methyl esters with methylation reagent "Meth-Prep 2". The methylation extract was then analysed by gas liquid chromatography method with flame ionisation detection (gas chromatograph Schimadzu G-2010-FID). The proportion of fatty acids content of the erythrocytes expressed as % of total fatty acids.

Muscle function and fitness tests

Grip strength was performed on both arms, with the maximum of 3 attempts recorded. Participants were seated with feet flat on floor, shoulder in neutral position with elbow bent at 90 degrees. Upper body muscular strength-endurance was measured using a 1-minute push-up test. Participants were asked to perform as many push-ups (knees on ground) as possible in 1 minute (American College of Sports Medicine 2010).

Lower body muscular endurance was measured using a 1-minute sit-stand test. The participant was asked to perform as many sit-stand movements as possible in 1 minute. Chair height was standardised at 43 cm height (American College of Sports Medicine 2010).

Sub-maximal aerobic capacity was measured using the modified Balke sub-maximal treadmill test. Seated blood pressure was measured before each assessment to ensure safety of exercise (Sharman & Stowasserb 2009). The test being completed when the individual had reached 85% of their estimated maximum heart rate (max HR) (est. maxHR = 220-age).

Statistical analysis

Baseline characteristics were compared between treatment types and stages of disease using independent samples t tests or ANOVA. Spearman's correlation coefficient was used assess the strength of bivariate associations, % time in moderate and vigorous activity were grouped together into one variable: % time in ≥ moderate activity. To assess the significance of age- and/or weight-adjusted associations between an outcome and a potential predictor, multivariable linear regression was used. Multivariable linear regression was used to model LBM as a function of various markers of fitness while also controlling for total body mass. For missing data, only those with full data sets were included in the models. The variables considered for inclusion in the model were those that were individually associated with LBM after adjusting for age and weight. Markers of fitness were added to the model sequentially, with the order determined by decreasing r-values. A predictor was only retained in the model if its coefficient was significantly different from zero at the 0.05 level. Adjusted R-squared was used to compare nested models. Models were also fitted that included interaction terms that explored the respective LCn-3 indices combined with fitness markers on LBM.

Results

Participants were recruited over a 15-month period (Oct 2011 – Jan 2013). A total of 135 women were initially screened for inclusion criteria. The major reasons for exclusion were >12 months post treatment completion and daily consumption of >1000 mg EPA and DHA combined. Forty-nine participants were eligible for the study and completed baseline assessment. Descriptive statistics of the population are shown in Table 1.

Age

Age was positively correlated with improved breast cancer related QOL ($r = 0.379$, $p = 0.007$), fatigue ($r = 0.311$, $p = 0.30$) and EPA ($r = 0.339$, $p = 0.026$), and negatively correlated with % of time in vigorous activity ($r = -0.342$, $p = 0.022$) and number of squats performed in 1-minute ($r = -0.363$, $p = 0.011$).

Table 1 Characteristics of participants

Characteristic (n = 49)	Value
Age in years (mean; SD)	48.6 ± 9.5
Race (n, %)	
—Caucasian	44(88)
—Asian	3(6)
—African	1(2)
—Asian Pacific Islander	1(2)
Anthropometric	**(mean; SD)**
Height (m)	1.65 ± 0.07
BMI (kgm-2)	26.6 ± 4
Body mass (kg)	73.1 ± 13.8
LBM (kg)	43.6 ± 5.6
Body fat %	39.5 ± 6.9
Waist (cm)	85.4 ± 11.1
Hip Girth (cm)	106.3 ± 9.2
CRP (n = 45; med; range)*	(0.1–10.1)
Total % RBC n-3 (n = 43)*	5.9 ± 1.6
% EPA	1.1 ± 0.5
% DHA	2.9 ± 0.9
Charaterstic of Disease (n; %)	
0-I	13 (26)
IIa	19 (28)
IIb-IIIa	17 (34)
Estrogen receptor + ve	39 (78)
HER-2 receptor + ve	12 (24)
Treatment variables (n; %)	
Had Chemotherapy	41 (92)
Taxane – Yes	37 (74)
Radiation therapy	
Tamoxifen	13 (26)
AI	20 (40)
None	16 (32)
Time since completion Rx	165 ± 107

AI–Aromatase inhibitors.
*Missing data.

Associations of diagnostic and treatment related variables

Compared to those diagnosed with earlier stage disease (0-IIa), those with later stage disease (IIb-IIIa) reported poorer results for BrCa related QOL (89.2 ±9.3 vs 79.3 ±15.7; p = 0.009), fatigue (130.5 ± 15.3 vs 113.3 ± 24.7; p = 0.006), total score for Greene climacteric scale (11.8 ± 6.8 vs. 17.5 ± 10.2; F = 5.308, p = 0.026), with specifically worse symptom scores reported for psychological, anxiety, depression and somatic fields (all $p < 0.05$). Stage of disease was not associated with any indices of body composition, LCn-3 or physical function.

Compared to those who did not have radiation therapy, DHA values (t = 2.904; p = 0.016) and LCn-3: LCn-6 (t = 3.06; p = 0.004) ratios were higher for those who underwent radiation therapy. Otherwise, radiation therapy was not associated with markers of body composition, QOL, dietary intake, LBM function, endurance or physical activity. Individuals taking tamoxifen tended to have lower EPA content compared to those taking AIs or no hormonal therapy (0.78% vs. 1.16% & 1.23%; F = 3.153, p = 0.054), however, there was no evidence to support an association between hormonal treatment and other markers of body composition, QOL, dietary intake, LBM function or physical activity.

Associations between LBM and dietary intake, inflammation, physical activity, markers of fitness and quality of life

LBM was positively correlated with daily intake of total energy (r = 0.301, p = 0.036) and protein (r = 0.464, p = 0.001), and negatively correlated with higher squat test results (r = −0.39, p = 0.006) (Table 2). However, after adjusting for weight and age, the only significant associations with LBM were % time spent in ≥ moderate intensity activity (ß: 0.228, p = 0.024), number of push-ups performed (ß: 0.343, p = 0.000) and treadmill stage completed (ß: 0.302, p = 0.001) (Table 2). CRP was positively correlated with body fat %, waist and hip however, these associations were no longer significant after controlling for total body weight (data not shown).

Associations between LCn-3 and anthropometric indices, inflammation & quality of life after breast cancer treatment

No significant correlations were identified between absolute LBM or % LBM for total RBC n-3, ratio of AA: EPA or % RBC content of EPA or DHA (Table 3). No significant relationships were found between any other anthropometric variables and n-3 related values.

No significant correlations were identified between CRP and erythrocyte LCn-3. No markers of body composition, CRP or indices of LCn-3 intake were significantly correlated with either measure of QOL.

Predictors of LBM in women soon after breast cancer

Number of push-ups, StageTM, and mod + vig activity were considered for inclusion in a weight-adjusted linear regression model for LBM (Table 2). Table 4 shows coefficients for the variables included in the final model. Table 4 also shows the value of adjusted R-squared obtained as each variable was successively added to the model. Mod + vig was not retained in the final model because the coefficient was not significantly different from zero (β=0.115, p = 0.177) in the presence of the other predictors. The model including weight, push ups and

Table 2 Associations between markers of absolute LBM and markers of LCn-3 intake, dietary intake, physical activity and fitness adjusted for weight & age n value

	Lean body mass (kg)				Body fat %			
	Unadjusted		Adjusted		Unadjusted		Adjusted	
	R	p-value	Standardise d B-coefficient	p-value	R	p-value	Standardise d B-coefficient	p-value
Total daily kJ intake	**0.301**	***0.036***	0.135	*0.132*	−0.82	*0.576*	−0.192	*0.065*
Total daily protein intake	**0.464**	***0.001***	0.144	*0.121*	0.04	0.787	−0.197	*0.068*
CRP*	0.258	*0.083*	−0.128	*0.238*	0.597	*0*	0.123	*0.343*
% time in sedentary activity*	0.167	*0.273*	0.053	*0.579*	0.23	*0.88*	−0.058	*−0.524*
% time in light activity*	−0.149	*0.329*	−0.104	*0.272*	0.156	*0.305*	0.125	*0.258*
% time in > moderate activity*	−0.041	*0.787*	**0.228**	***0.024***	−0.466	*0.001*	**−0.275**	***0.015***
Push up (in 1-min)*	−0.045	*0.760*	0.343	***0.000***	−0.671	*0*	**−0.457**	***0***
Squats (in 1-min)*	**−0.39**	***0.006***	0.044	*0.71*	−0.454	*0.001*	−0.098	*0.439*
Stage of treadmill completed	−0.047	*0.746*	**0.302**	***0.001***	−0.575	*0*	**−0.39**	***0***
FACT-B + 4	−0.13	*0.373*	0.038	*0.699*	−0.146	*0.316*	−0.009	*0.936*
FACT-F	−0.128	*0.381*	0.023	*0.813*	−0.133	*0.362*	−0.002	*0.982*

% time in activity: Accelerometry; % time in > moderate activity: moderate and vigorous activity grouped together; Stage of treadmill completed: at which 85% of estimated HRmax was reached; *Reduced data: CRP: n = 46; Accelerometer data: n = 45; Push-ups: n = 48; Squats: n = 48.

StageTM explained 76.4% of the variation in absolute LBM (Table 4).

Interactions of physical activity and indices of LCn-3 intake on markers of LBM function

The number of push-ups performed was positively correlated with time spent in ≥ moderate intensity activity (r = 0.467; p = 0.001), total n-3 levels (r = 0.385; p = 0.012) and DHA levels (r = 0.517, p = 0.000) (Table 5). The correlation with total n-3 levels was no longer statistically significant after adjusting for DHA. DHA maintained a significant association after adjusting for age, weight, LBM and % time > mod activity (β=0.399, p = 0.001) % ≥. Mod activity remained a significant predictor (F-Test: 8.95, p = 0.005) of the number of push-ups performed in one minute after adjusting for DHA, age, weight and LBM. There were no significant interactions between RBC LCn-3 and time spent in any intensity of activity for any of the regression models of physical function (data not shown).

Discussion

This paper reports a positive relationship between LBM (adjusted for total weight) and physical function represented by

Table 3 Univariate associations between indices of erthrocyte LCn-3 s and markers of body composition, inflammation and quality of life (n = 43)

	Total n-3		EPA		DHA		AA/EPA	
	r	*p-value*	r	*p-value*	r	*p-value*	r	*p-value*
Weight (kg)	0.083	*0.595*	0.249	*0.107*	−0.088	*0.576*	−0.232	0.134
LBM (kg)	0.093	*0.554*	0.156	*0.319*	−0.27	0.864	−0.159	*0.309*
Body fat %	0.037	*0.816*	0.222	*0.153*	−0.107	*0.493*	−0.181	*0.245*
Waist (cm)	0.123	*0.431*	0.280	*0.069*	−0.061	*0.697*	−0.167	*0.284*
Hip (cm)	0.055	*0.728*	0.280	*0.069*	−0.141	*0.366*	**−0.313**	***0.041***
CRP (mmol/L)	0.035	*0.822*	0.183	*0.241*	−0.42	*0.791*	−0.224	*0.149*
FACT-B + 4	−0.063	*0.689*	0.039	*0.804*	−0.064	*0.683*	−0.007	*0.962*
FACT-F	−0.129	*0.411*	0.040	*0.797*	−0.137	*0.382*	−0.026	*0.868*

LBM: Lean Body mass; CRP: C-reactive protein; EPA: eicosapentanoic acid; DHA: docosahexanoic acid; FACT-B + 4: Quality of life with breast related items; FACT-F: Fatigue.

Table 4 Best predictors of LBM post-treatment using hierarchical regression

Predictor	Regression coefficient* (95% CI)	p-value**	Adjusted R^{2t}
Weight	0.948	0.000	0.634
Stage Tmill completed	0.225	0.007	0.713
No. push ups (1 min)	0.275	0.002	0.764

*Regression coefficients taken from final model including: body mass, number push-ups performed, treadmill stage reached.
**Significance of the individual predictor within the final model.
[t]Denotes value reported as each variable was added into the model in the order: body mass, stage tmill completed then no. push ups
CI = confidence interval.

the % time spent in ≥ moderate intensity physical activity, stage achieved on sub-maximal treadmill test and number of push-ups completed. To the authors' knowledge, this is the first study to determine associations between physical function and body composition in women who have completed treatment for breast cancer.

Our results agree with previous cross-sectional and prospective cohort studies, which have shown that decreasing physical activity levels are associated with greater adverse body composition change,(Irwin et al. 2003; Irwin et al. 2005) while dietary measures(Demark-Wahnefried et al. 2001) have been less predictive of these changes. The findings relating to the influence of chemotherapy on LBM agree with two previous studies (Campbell et al. 2007; Winters-Stone et al. 2009) but are in contrast to five studies that have shown a greater decrease in LBM after chemotherapy (Cheney et al. 1994; Demark-Wahnefried et al. 1997; Demark-Wahnefried et al. 2001; Gordon et al. 2011; Kutynec et al. 1999). In addition, Prado et al. reported that individuals with chemotherapy toxicity had a greater risk of sarcopenia (Prado et al. 2009). Differences in our results may be due to the cross-sectional nature of the study. Previously published data sets indicating LBM change after chemotherapy and hormonal therapies were prospective in nature (Cheney et al. 1994; Demark-Wahnefried et al. 1997; Demark-Wahnefried et al. 2001; Gordon et al. 2011; Kutynec et al. 1999) and were able to see trends over time.

No associations were found between erythrocyte LCn-3 and markers of body composition. Recent studies in populations during and post-chemotherapy treatment have indicated a positive relationship between skeletal muscle mass and plasma phospholipid LCn-3 content (Murphy et al. 2010; Murphy et al. 2011), however these participants experienced significant and rapid muscle loss during treatment. After early stage breast cancer treatment, the rate and magnitude of muscle loss experienced is not typically as high as when compared to more advanced staged cancers (Mcdonald et al. 2011; Murphy et al. 2010). As a result, our results are comparable to metabolic/obese populations undergoing similar body composition change (Krebs et al. 2006; Noreen et al. 2010; Storlien et al. 2001).

Total body mass, push-ups performed in one-minute, and stage completed on treadmill remained in the final model accounting for 76% of the variation in LBM. These results are of interest as they indicate an association with physical function and healthier body composition. Specifically, the strength of association with number of push-ups/minute as opposed to squats may indicate the importance of whole body resistance training to maintain or achieve a higher LBM and lower fat mass. A decrease in sports/recreational exercise has been previously associated with an increase in adiposity however, LBM change was not reported (Irwin et al. 2005). It is possible that those who performed more push-ups due to an increase in relevant exercise training may also be more conscientious in regards to dietary intake, however no association was found in this study.

Both erythrocyte DHA and EPA content were associated with markers of physical function, surprisingly in positive and negative directions, respectively. DHA was strongly and independently associated with the ability to perform push-ups, while erythrocyte EPA content was negatively associated with squats performed. In addition, assessing predictive models for push-up performance, when%time ≥ moderate physical activity was added to the DHA model, a greater effect was seen. In contrast, EPA content remained significantly negatively associated with squats performed. Previous studies have indicated an increase in muscle protein synthesis (Smith et al. 2011a;

Table 5 Correlations between measures of physical function and LCn-3 content of erythrocytes

Physical function*	% time>mod		EPA		EPA adj**		DHA		DHA adj.**	
	r	p-value	r	p-value	β	p-value	r	pcs-value	β	p-value
Push ups	0.467	0.001	0.072	0.648	0.212	0.118	0.517	0.000	0.399	0.001
Squats	0.110	0.479	−0.338	0.029	-0.268	0.041	0.153	0.333	-0.37	0.776
Handgrip	-0.068	0.663	0.083	0.603	-0.144	0.340	0.109	0.492	0.099	0.482
Treadmill	0.224	0.138	-0.11	0.493	0.13	0.929	0.267	0.083	0.147	0.277

*Push ups: performed in one-minute; Squats: performed in one-minute; Stage of treadmill completed: at which 85% of estimated HRmax was reached.
% time > mod: moderate & vigorous data combined; EPA: eicosapentanoic acid; DHA: docosahexanoic acid.
**Fully adjusted model included: weight, age, % time > mod activity & LBM. Correlation coefficient (β)

Smith et al. 2011b) and peak torque development (Rodacki et al. 2012) after supplementation of LCn-3 s was combined with an anabolic stimulus. In advanced cancer populations, EPA LCn-3 supplementation (often in conjunction with a protein-rich supplement) has been associated with improvements in physical function (Moses et al. 2004) and strength (Fearon et al. 2006), while EPA and DHA LCn-3 + NSAIDs have been shown to improve handgrip strength (Cerchietti et al. 2007). Our results both agree and disagree with the previous literature base with no clear reason for the opposing directions for the associations between physical function, DHA and EPA. Further investigation into LCn-3 and physical activity interactions are required.

Our population compared favourably with larger cohorts for body composition, (Chlebowski et al. 2002; Irwin et al. 2005) education level, (Irwin et al. 2005) however the exclusion of those with a diagnosed chronic disease (T2DM or CVD) and those who could not participate in moderate physical activity, may have led to our participants being younger and more physically active than the general breast cancer population.

Conclusion

This is the first study to report that higher weight adjusted LBM is associated with greater upper body strength-endurance and aerobic fitness in women after completion of treatment for breast cancer. Further research is required to elucidate LCn-3-exercise interactions.

Competing interest

All capsules for the intervention were provided by Blackmores Pty Ltd; no intellectual input regarding study design, data collection, analysis or write up was given.

Authors' contribution

CM-Contributed to study design, carried out intervention phase, data collection & analysis, write up; JB-Study design, input into analysis and interpretation, intellectual input for write up; SC- study design, analysis and interpretation, write up; MW- Statistician, analysis and write up. All authors read and approve the final manuscript.

Acknowledgements

Funding for the blood analyses, exercise equipment other costs were provided by a grant from the Wesley Research Institute (Grant no. 1034).

Received: 5 November 2013 Accepted: 8 November 2013
Published: 19 December 2013

References

American College of Sports Medicine (2010) ACSM's Guidelines for Exercise Testing and Prescription, 8th edn. Wolters Kluwer/Lippincott Williams & Wilkins, Philadelphia

Campbell KL, Lane K, Martin AD et al (2007) Resting Energy Expenditure and Body Mass Changes in Women During Adjuvant Chemotherapy for Breast Cancer. Cancer Nursing 30:95–100

Cella D, Tulsky D, Gray G et al (1993) The Functional Assessment of Cancer Therapy scale: Development and validation of the general measure. J Clin Oncol 11:570–579

Cerchietti LCA, Naviganteac AH, Castroa MA (2007) Effects of Eicosapentaenoic and Docosahexaenoic n-3 Fatty Acids From Fish Oil and Preferential Cox-2 Inhibition on Systemic Syndromes in Patients With Advanced Lung Cancer. Nutr Cancer 59:14–20

Cheney CL, Mahloch J, Freeny P (1994) Computerised tomography assessment of women with weight changes associated with adjuvant treatment for breast cancer. Am J Clin Nutr 66:141–146

Chlebowski RT, Aiello E, Mctiernan A (2002) Weight Loss in Breast Cancer Patient Management. J Clin Oncol 20:1128–1143

Courneya KS, Segal RJ, Mackey JR et al (2007) Effects of Aerobic and Resistance Exercise in Breast Cancer Patients Receiving Adjuvant Chemotherapy: A Multicenter Randomized Controlled Trial. J Clin Oncol 25:4396–4404

Demark-Wahnefried W, Hars V, Conaway MR et al (1997) Reduced rates of metabolism and decreased physical activity in breast cancer patients receiving adjuvant chemotherapy. Am J Clin Nutr 65:1495–1501

Demark-Wahnefried W, Peterson BL, Winer EP et al (2001) Changes in Weight, Body Composition, and Factors Influencing Energy Balance Among Premenopausal Breast Cancer Patients Receiving Adjuvant Chemotherapy. Journal of Clinical Oncology 19:2381–2389

Dewey A, Baughan C, Dean T, Higgins B, Johnson I (2007) Eicosapentaenoic acid (EPA, an omega-3 fatty acid from fish oils) for the treatment of cancer cachexia. The Cochrane database of systematic reviews (1469-493X) 1:CD004597

Fearon KCH, Barber MD, Moses AG et al (2006) Double-Blind, Placebo-Controlled, Randomized Study of Eicosapentaenoic Acid Diester in Patients With Cancer Cachexia. Journal of Clinical Oncology 24:3401–3407

Fearon KCH, Von Meyenfeldt MF, Moses AGW et al (2003) Effect of a protein and energy dense n-3 fatty acid enriched oral supplement on loss of weight and lean tissue in cancer cachexia: a randomised double blind trial. Gut 52:1479–1486

Francini G, Petrioli R, Montagnani A et al (2006) Exemestane after tamoxifen as adjuvant hormonal therapy in postmenopausal women with breast cancer: effects on body composition and lipids. Br J Cancer 95:153–158

Gordon AM, Hurwitz S, Shapiro CL et al (2011) Premature ovarian failure and body composition changes with adjuvant chemotherapy for breast cancer. Menopause 18:1244

Healy LA, Ryan AM, Carroll P et al (2010) Metabolic Syndrome, Central Obesity and Insulin Resistance are Associated with Adverse Pathological Features in Postmenopausal Breast. Clinical Oncology 22:281–288

Ibrahim EM, Al-Homaidh A (2011) Physical activity and survival after breast cancer diagnosis: meta-analysis of published studies. 28(3):65–753. doi:10.1007/s12032-010-9536-x. Epub 2010 Apr 22

Irwin ML, Alvarez-Reeves M, Cadmus L et al (2009) Exercise Improves Body Fat, Lean Mass, and Bone Mass in Breast Cancer Survivors. Obesity 17:1534–1541

Irwin ML, Crumley D, Mctiernan A et al (2003) Physical activity levels before and after a diagnosis of breast carcinoma. Cancer 97:1746–1757

Irwin ML, Mctiernan A, Baumgartner RN et al (2005) Changes in body fat and weight after a breast cancer diagnosis: influence of demographic, prognostic, and lifestyle factors. Journal of Clinical Oncology 23:774–782

Krebs JD, Browning LM, Mclean NK et al (2006) Additive benefits of long-chain n-3 polyunsaturated fatty acids and weight-loss in the management of cardiovascular disease risk in overweight hyperinsulinaemic women. Int J Obes 30:1535–1544

Kutynec CI, Mccargar L, Barr SI et al (1999) Energy balance in women with breast cancer during adjuvant treatment. Journal of the American Dietetic Association 99:1222–1227

Martin GS (2004) The interviewer-administered, open-ended diet history method for assessing usual dietary intakes in clinical research: relative and criterion validation studies. In: University of Wollongong Thesis Collection. University of Wollongong, Wollongong

Mcdonald C, Bauer JM, Capra S et al (2013a) The Muscle mass, Omega-3, Diet, Exercise and Lifestyle (MODEL) study - study protocol for a randomised controlled trial for women who have completed breast cancer treatment. BMC Cancer, In Press

Mcdonald CK, Bauer JD, Capra S (2011) Body Composition and Breast Cancer- the Role of Lean Body Mass. Cancer Forum 35:102–106

Mcdonald CK, Bauer JD, Capra S (2013b) Omega-3 fatty acids and changes in LBM – alone or in synergy for better muscle health? A Review. Canadian Journal of Physiology and Pharmacology, in press

Mcneely ML, Campbell KL, Rowe BH et al (2006) Effects of exercise on breast cancer patients and survivors: a systematic review and meta-analysis. CMAJ: Canadian Medical Association Journal 175:34–41

Montagnani A, Gonnelli S, Cadirni A et al (2008) The effects on lipid serum levels of a 2-year adjuvant treatment with exemestane after tamoxifen in postmenopausal women with early breast cancer. European Journal of Internal Medicine 19:592–597

Moses AW, Slater C, Preston T et al (2004) Reduced total energy expenditure and physical activity in cachectic patients with pancreatic cancer can be modulated by an energy and protein dense oral supplement enriched with n-3 fatty acids. Br J Cancer 90:996–1002

Mourtzakis M, Bedbrook M (2009) Muscle atrophy in cancer: a role for nutrition and exercise. Applied Physiology, Nutrition and Metabolism 34:950–956

Murphy RA, Mourtzakis M, Chu QS et al (2010) Skeletal Muscle Depletion Is Associated with Reduced Plasma (n-3) Fatty Acids in Non-Small Cell Lung Cancer Patients. The Journal of Nutrition 140:1602–1606

Murphy RA, Mourtzakis M, Chu QSC et al (2011) Nutritional intervention with fish oil provides a benefit over standard of care for weight and skeletal muscle mass in patients with nonsmall cell lung cancer receiving chemotherapy. Cancer 117:1775–1782

Murphy RA, Mourtzakis M, Mazurak VC (2012) n-3 polyunsaturated fatty acids: the potential role for supplementation in cancer. Current opinion in clinical nutrition and metabolic care 15:246

Newman AB, Kupelian V, Visser M et al (2006) Strength, But Not Muscle Mass, Is Associated With Mortality in the Health, Aging and Body Composition Study Cohort. The Journals of Gerontology Series A: Biological Sciences and Medical Sciences 61:72–77

Noreen Eric E, Sass Michael J, Crowe Megan L, Pabon Vanessa A, Brandauer J, Averill Lindsay K (2010) Effects of supplemental fish oil on resting metabolic rate, body composition, and salivary cortisol in healthy adults. Journal of the International Society of Sports Nutrition, ISSN 1550-2783 7(1):31

Pedersen BK, Febbraio MA (2012) Muscles, exercise and obesity: skeletal muscle as a secretory organ. Nature reviews Endocrinology 8:457

Pierce BL, Ballard-Barbash R, Bernstein L et al (2009) Elevated Biomarkers of Inflammation Are Associated With Reduced Survival Among Breast Cancer Patients. J Clin Oncol 27:3437–3444

Prado CMM, Baracos VE, Mccargar LJ et al (2009) Sarcopenia as a Determinant of Chemotherapy Toxicity and Time to Tumor Progression in Metastatic Breast Cancer Patients Receiving Capecitabine Treatment. Clinical Cancer Research 15:2920–2926

Ries A, Trottenberg P, Elsner F et al (2012) A systematic review on the role of fish oil for the treatment of cachexia in advanced cancer: an EPCRC cachexia guidelines project. Palliat Med 26:294–304

Rodacki CL, Rodacki AL, Pereira G et al (2012) Fish-oil supplementation enhances the effects of strength training in elderly women. The American Journal of Clinical Nutrition 95:428–436

Ruiz JR, Sui X, Lobelo F et al (2008) Association between muscular strength and mortality in men: prospective cohort study. British Medical Journal (Clinical Research Ed) 337:a439–495

Schmitz KH, Ahmed RL, Hannan PJ et al (2005) Safety and Efficacy of Weight Training in Recent Breast Cancer Survivors to Alter Body Composition, Insulin, and Insulin-Like Growth Factor Axis Proteins. Cancer Epidemiology, Biomarkers & Prevention 14:1672–1680

Sharman A, Stowasserb M (2009) Australian Association for Exercise and Sports Science Position Statement on Exercise and Hypertension. Journal of Science and Medicine in Sport 12:252–257

Sheean PM, Hoskins K, Stolley M (2012) Body composition changes in females treated for breast cancer: a review of the evidence. Breast Cancer Res Treat 135:663–680

Smith GI, Atherton P, Reeds DN et al (2011a) Dietary omega-3 fatty acid supplementation increases the rate of muscle protein synthesis in older adults: a randomized controlled trial. Am J Clin Nutr 93:402–412

Smith GI, Atherton P, Reeds DN et al (2011b) Omega-3 polyunsaturated fatty acids augment the muscle protein anabolic response to hyperinsulinaemia-hyperaminoacidaemia in healthy young and middle-aged men and women. Clinical science (London, England: 1979) 121:267–278

Storlien LH, Robertson RM, Hill JO et al (2001) Role of weight loss and polyunsaturated fatty acids in improving metabolic fitness in moderately obese, moderately hypertensive subjects. J Hypertens 19:1745–1754

Van Der Meij BS, Van Bokhorst-De Van Der Schueren MA, Langius JA et al (2011) n – 3 PUFAs in cancer, surgery, and critical care: a systematic review on clinical effects, incorporation, and washout of oral or enteral compared with parenteral supplementation. The American Journal of Clinical Nutrition 94:1248–1265

Van Londen G, Perera S, Vujevich K et al (2011) The impact of an aromatase inhibitor on body composition and gonadal hormone levels in women with breast cancer. Breast Cancer Res Treat 125:441–446

Winters-Stone KM, Nail L, Bennett JA et al (2009) Bone health and falls: fracture risk in breast cancer survivors with chemotherapy-induced amenorrhea. Oncology Nursing Forum 36:315–325

doi:10.1186/2193-1801-2-681
Cite this article as: McDonald *et al.*: **Muscle function and omega-3 fatty acids in the prediction of lean body mass after breast cancer treatment.** *SpringerPlus* 2013 **2**:681.

4.2 Results

From the theoretical model, we hypothesized that treatment (Sx, RTx and CTx) was associated with increased inflammation, early menopause and decreased physical activity. In turn, these factors are hypothesized to be associated with greater loss of LBM, and increases in body fat% and/or body weight. In contrast, previous research has indicated the positive association between AI usage and LBM gains. The results below are included to compare relevant data to the existing evidence substantiating our theoretical model (Figure 4.2).

4.2.1 Associations between proposed outcomes and breast cancer treatment.

Forty-one (92%) of participants underwent chemotherapy, while 33 (67%) had radiotherapy. Compared to those who did not receive chemo, there were no differences in any marker of body composition, inflammation, markers of physical function or time spent in >moderate intensity activity (all $p>0.05$). At baseline, quality of life scores tended to be greater for those who had not undergone chemotherapy, compared to those who had (113.6 ± 8.5 vs. 106.22 ± 16.4, $p=0.077$).
No additional significant relationships were noted when comparing those who underwent radiotherapy with those who had no radiotherapy.
Further analyses investigating treatment type and stage of disease revealed that those who were diagnosed with later stage disease (Stage IIB & IIIA) were more likely to undergo chemotherapy than those with earlier stage disease (Stage 0 to IIA) (Chi-square: 100% vs 75.0%, Fisher's Exact: $p=0.038$). In the published manuscript we reported an increase in fatigue and decreased quality of life for those with later stage disease. However, stage of disease was not associated with any measure of body composition, physical function, or CRP (all $P>0.05$).

4.2.2 Associations between intermediary outcomes and body composition measures

Aromatase Inhibitors and body composition

No significant differences were observed in body composition when comparing those on aromatase inhibitors and those on tamoxifen or no hormonal treatment (data not shown, all $p<0.05$). In addition, no association between hormonal treatment and CRP or FACT-B scores (all $p>0.05$, data not shown) were noted.

Physical activity and body composition

Physical activity measured objectively through 7-day accelerometry and indirectly through tests of physical function, (1-min push up, squat, treadmill test) were strongly associated to higher LBM after adjustment for weight and age. Body fat% was significantly associated physical activity

(reported in the manuscript). Furthering this, we observed an inverse association between physical activity and: waist girth (β: -1.157, p=0.001) and hip girth (β: -0.857, p=0.021).
When assessed as tertiles of physical activity variables: time spent in ≥moderate intensity activity, number of push-ups performed and stage reached in the treadmill test, all three variables had a significant linear relationship with LBM. Such that more physical activity or greater physical function was related to greater adjusted LBM (all p<0.05). However, both 2nd (3.4+0.59%) and 3rd (6.2+2.2%) tertiles of %time spent in >moderate intensity activity had significantly greater adjusted LBM than the first (1.2+0.6%) tertile (absolute values of LBM (kg): 44.9+0.7kg & 44.3kg+0.7 vs. 42.03+0.7kg vs, respectively) (Figure 4.1). For push-ups (1st: 0.4+0.79, 2nd: 5.6+2.7, 3rd: 20.5+9.0) and StageTM (1st: 9.6+1.4 2nd: 12.0+0.00, 3rd: 13.8+0.9), the highest tertile of each had significantly greater adjusted LBM than the 1st or 2nd tertiles (Figure 4.1). For body fat%, the relationships were equivalent and significant albeit in the inverse direction (data not shown).

CRP and body composition

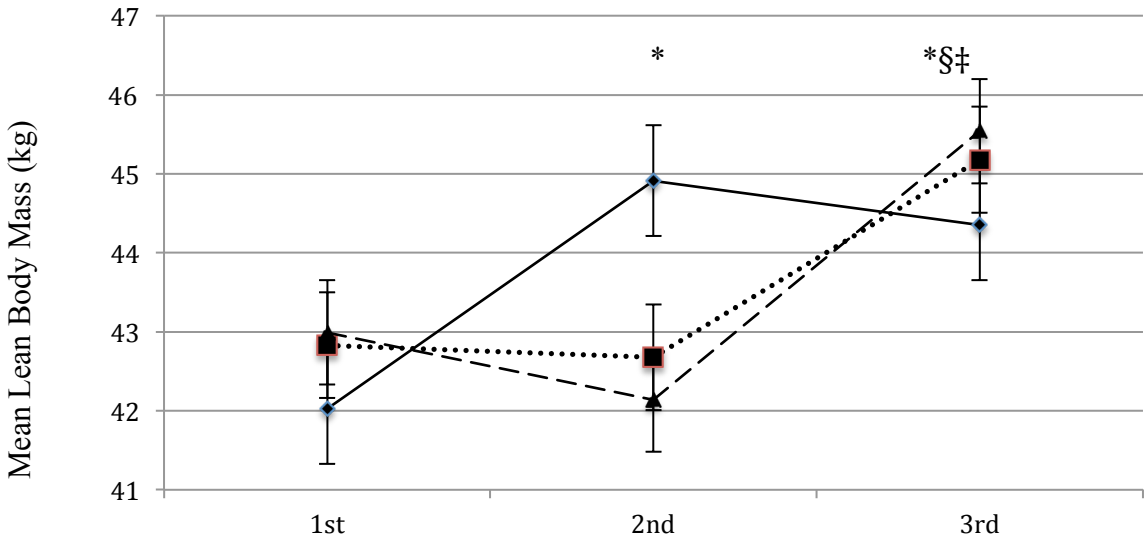


Figure 4.1 LBM for tertiles of time spent in >moderate intensity activity, push-ups performed and stage of treadmill completed (n=49)

X-values indicate tertile grouping. Covariates included in the model: body weight, height and age. Estimated Marginal Means of LBM indicated on Y-Axis.
Full line (—):tertiles of %time spent in >moderate intensity activity (ModVig); Dashed line (---): tertiles of Stage reached on Treadmill (StageTM); Dotted line (•••): tertiles of push ups performed in 1-min (Push ups)
*Significantly different from 1st tertile of ModVig, p<0.05
§Significantly different from 1st and 2nd tertiles of StageTM, p<0.05
‡Significantly different from 1st and 2nd tertiles of Push ups, p<0.05

While our main results indicated no effect for CRP on LBM we investigated associations with tertiles of CRP. There was an overall trend for higher CRP values being associated with lower LBM (F=2.89, p=0.067) and higher body fat% (F=3.5, p=0.038) after adjusting for weight and age. In addition, compared to the first tertile, the third tertile had significantly lower adjusted LBM (45.26+0.96kg vs. 41.71+0.98kg, p=0.021) and greater body fat% (36.5+1.4% vs. 42. 1+1.4kg,

p=0.011). However, after adjustment for height, linear (p for trend=0.244 & 0.197) relationships were no longer significant for LBM or body fat%, respectively. No significant relationships for CRP were noted for waist and hip girths.

Menopausal status in body composition, inflammation and QOL

Of the 49 women, 24 (46.9%) were classified as post-menopausal. No associations for menopausal status (pre-, peri- and postmenopausal) were found for LBM, body fat%, waist or hip girths. When menopausal states were compared for CRP, no differences were noted for pre- (n=6) peri- (n=13) or postmenopausal (n=21) women (median: 1.5mg/L 95%CI: 0.98 to 6.7; 0.7mg/L 95%CI: 0.22 to 3.2 & 0.9mg/L, 95%CI: 0.9 to 2.5, respectively, all p>0.2). In contrast, no differences were noted for quality of life scores or erythrocyte content of EPA or DHA (all P<0.05 for linear trend and between groups). However, due to the small number of premenopausal women, these results should be interpreted with caution.

Erythrocyte LCn-3 and associations with body composition, inflammation and QOL

Associations between EPA, DHA and LBM presented as contrasting, albeit non-significant patterns (Table 4.1). Numerically the tertiles indicated a negative and positive association with LBM for EPA and DHA, respectively. However, the small magnitude of difference was not clinically significant.

Furthermore for tertiles of EPA, no significant associations were found for body fat%, waist, hip, CRP or FACT-B scores (Table 4.1). For DHA, a non-significant trend was observed for hip circumference, such that a lower hip girth tended to be associated with a greater % of DHA in the erythrocytes (p=0.062). Post-hoc analyses revealed that compared to the tertile 1, the 2nd tertile of DHA intake had a significantly lower hip girth (p=0.022), while the highest tertile was only numerically lower and not statistically significant.

4.2.3 Discussion of additional baseline findings

The influence of breast cancer diagnosis and treatment variables on intermediary and body composition outcomes

There was no effect for time since diagnosis or treatment completion on any marker of body composition. In addition, we found that neither chemotherapy nor radiotherapy had an influence on performance of physical activity, quality of life or markers of inflammation in a population of women who had completed treatment for breast cancer in the last 12 months. Similarly, stage of disease did not influence any of these measures except for quality of life and fatigue. Chemotherapy has been associated with reduced physical activity previously (Demark-Wahnefried et al. 1997) and a combination of decreased activity and chemotherapy being conducive to greater body weight gain compared to those who maintain their activity (Irwin et al. 2005)

TABLE 4.1 THE EFFECT OF ERYTHROCYTE LCN-3 CONTENT (TERTILES) AND CROSS SECTIONAL ASSOCIATIONS OF BODY COMPOSITION, INFLAMMATION AND QUALITY OF LIFE.

	%EPA in RBC				%DHA in RBC			
	Tertile 1	Tertile 2	Tertile 3	P trend*	Tertile 1	Tertile 2	Tertile 3	P trend*
%EPA/DHA[1]	0.68 (0.17)	0.99 (0.07)	1.6 (0.45)	0.000	1.96 (0.6)	2.9 (0.18)	3.7 (0.5)	0.000
LBM (kg)	44.1 (0.7)	43.7 (0.7)	42.8 (0.7)	0.439	42.9 (0.7)	44.3 (0.7)	44.6 (0.8)	0.247
Body fat%	38.4 (1.0)	39.3 (1.1)	40.1 (1.1)	0.526	40.7[b] (1.1)	39.0 (1.1)	38.1[b] (1.1)	0.241
Waist (cm)	85.5 (0.8)	85.0 (0.9)	84.5 (0.9)	0.726	84.8 (0.9)	86.3 (0.9)	84.8 (0.9)	0.374
Hip (cm)	105.7 (0.78)	106.0 (0.9)	108.9 (0.8)	0.594	108.3[a] (0.8)	105.5[a] (0.8)	106.5 (0.8)	0.062
CRP (mg/L)	1.59 (0.47)	1.2 (0.5)	2.2 (0.5)	0.453	2.1 (0.5)	0.8[b] (0.5)	2.1[b] (0.5)	0.143
FACT-B	110.2 (3.5)	102.4 (3.8)	111.7 (3.7)	0.183	110.8 (3.9)	106.8 (4.0)	109.5 (4.0)	0.765

[1]For %EPA tertiles, value for EPA is shown, for %DHA tertiles, value for DHA is shown.
*Indicates linear trend for tertiles univariate analysis of covariance. All values are estimated marginal means, covariates included: weight, age, height.
[a]: indicates significantly different to Tertile 1, p=0.022. [b]: Indicates a non-significant trend for difference compared to Tertile 1, p<0.1.

However, our results agree with a recent long term follow up of breast cancer survivors that indicated no difference in quality of life for those who were treated with chemotherapy or not (Hsu et al. 2013).

Chemotherapy (Aslani et al. 1999, Prado et al. 2009) and radiotherapy (Bower et al. 2009) have previously been associated with increases in markers of inflammation in those with cancer. Bower et al (2009) noted that radiation induced elevations in CRP and IL-6 were related to increases in fatigue in breast and prostate cancer survivors (Bower et al. 2009). These previous findings may explain our observation that those with later stage disease all underwent chemotherapy had increased fatigue and poorer quality of life. However, we found no difference between groups for concentration of high sensitivity (hs)-CRP.

.

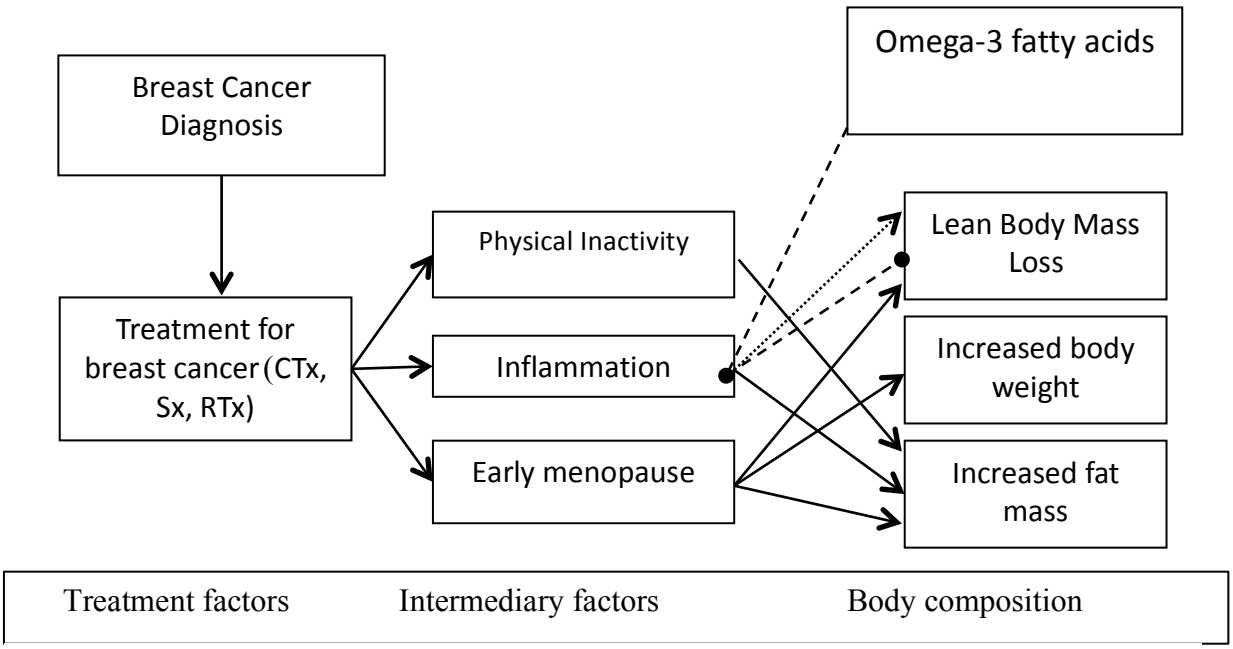


Figure 4.2 Theoretical framework for body composition change after treatment for breast cancer.

Connecting lines indicate an association established in previous observational studies. Solid line (-): Research conducted in breast cancer populations; Dotted line (……): Research conducted in non-breast cancer populations; Arrow: Agonistic relationship; Solid circle: Antagonistic relationship. Dashed line (---): Proposed relationship under investigation.

Further analysis of physical function and effect on body composition

A threshold association was noted for cross-sectional body composition and markers of physical activity and function. Those who achieved 20.4 (95%CI: 15 to 25) push ups in 1-minute, reached stage 13.8 (95%CI: 13.4 to 14.5) or spent more than 3.5% (95%CI: 3.1 to 7.5) of their time performing ≥moderate intensity activity were more likely to have a greater weight, age and height adjusted LBM. Previous studies in breast cancer have not assessed the relationship between physical function and LBM outcomes. However, a number of exercise interventions have shown that increasing strength and cardiorespiratory fitness through training can result in LBM increase (Schmitz, Ahmed, et al. 2005, Herrero et al. 2006, Fernández-Lao et al. 2013, Irwin, Alvarez-Reeves, et al. 2009), but not in all cases (Schmitz et al. 2009, Demark-Wahnefried et al. 2008, DeNysschen et al. 2011). In a general female population (N=1443), physical activity participation was associated with increased muscle strength, and in turn, increased strength was associated with increased absolute LBM (Rolland et al. 2004). Thus, our data agree with the well-established links between participation in physical activity and increased LBM.

Effect of early menopause on breast cancer outcomes

We found no significant relationships between menopausal state and intermediary or body composition outcomes. Previous prospective research has indicated that women who experience early onset of menopause as a result of treatment are likely to gain more weight (Goodwin et al. 1999, Niraula et al. 2012). A previous review of age, menopausal status and quality of life indicates that younger women typically experience lower quality of life after treatment (Howard-Anderson et al. 2012). One of the suggested drivers of this relationship is the onset of early menopause and fertility concerns (Howard-Anderson et al. 2012), however there is a dearth of research explaining quality of life and physical function differences between menopausal states. Our preliminary data indicates that menopausal status has little influence, although the study limited by it's cross sectional nature.

Effect of erythrocyte EPA and DHA concentrations

Tertiles of neither EPA nor DHA were related to inflammation in our population. Previous epidemiological research has been inconsistent when describing the relationship between incorporation of LCn-3 into plasma tissue, and its association with lower concentrations of inflammatory markers (Calder 2012, Pischon et al. 2003). Observational research in breast cancer survivors reported that increased CRP was associated with higher fatigue and lower concentrations of LCn-3 compared to LCn-6 ratios (Alfano et al. 2012). Further analysis of our data did not show a significant association with CRP for ratios of LCn-3 to LCn-6. It is possible that these results were limited by our relatively low sample size. On the other hand we observed a generally low CRP value in our participants (1.6 $\pm$2.2mg/L) compared to the study by Alfano et al (4.4$\pm$8.6) (Alfano et al. 2012), thus a larger range in CRP may have made it's effect more pronounced.

We noted that increasing %DHA in the erythrocytes tended to be associated with decreased hip girth, with the 2nd tertile of %DHA had a significantly lower hip girth than the lowest tertile. DHA has been reported previously to be associated with changes in measures of body fat% (Munro and Garg 2012). Munro et al reported a significant correlation between DHA and body weight and body fat loss (Munro and Garg 2012), while a number of other studies have shown that LCn-3 supplementation is related to greater loss in fat mass (Couet et al. 1997, Kabir et al. 2007, Noreen et al. 2010). However, we did not observe any other significant results for different markers of adiposity.

Limitations

Originally, a recruitment rate of two to three participants per week was estimated given clinical data from the oncologists referring participants to the trial. After 19 months of recruitment, only ~30% of the proposed sample size was initiated. Efforts were made to contact local chemotherapy units in

Brisbane based hospitals, a larger list of oncologists, breast cancer and women's centres, social media, public radio advertising and national research recruitment centres. Despite these strategies, our greatest limitation to recruiting participants were geographical barriers (women living >60 minutes drive away from the Wesley Research Institute), and that many women were excluded as they had completed treatment more than 12 months prior to recruitment. These criteria were considered necessary, however the lower than projected number of participants is likely to have reduced the ability to identify relationships both at baseline and as a result of the intervention.

4.3 Summary of findings at baseline

In a population of women who have been recently treated for breast cancer, LBM is predominantly predicted by a combination of body weight, estimated cardiorespiratory fitness and upper, but not lower body strength-endurance. Physical activity measured objectively was related to LBM after adjustment for weight and age, however was not significant when controlling for cardiorespiratory fitness and upper body strength-endurance. Furthermore, a threshold effect of performance over those three measures in relation to weight adjusted LBM. In essence, LBM is likely to be greater in women who engage in physical activity that enhances or maintains a higher level of endurance and upper body strength-endurance. The fact that the relationship of LBM and upper body strength-endurance differs from lower body strength-endurance may indicate two things: 1) that whole body strength training is necessary for maintenance of greater LBM, and/or 2) those who engage in exercise training that elicits greater upper body strength are a population who are likely to have greater LBM.

We did not see a significant effect for LCn-3 on any marker of body composition, quality of life or inflammation. This data differs from that found in people with cancer undergoing more intense treatment for later stage disease, however it matches the mixed findings from healthy or chronically diseased populations. Since women who have completed breast cancer treatment are metabolically more similar to those chronically diseased, our findings are not surprising.

These findings are limited by their cross-sectional nature and the shortfall in recruitment that we experienced. Chapter 5 will describe findings from our intervention that investigates the effect of manipulating physical activity and LCn-3 consumption in this population of women who have completed treatment for breast cancer.

Chapter 5 – Intervention Results

Section 1 is a submitted manuscript that reports the primary and secondary findings of the thesis at 12 and 24 weeks. The intervention indicated the LCn-3 supplementation and a 12-week lifestyle program have a synergistic effect in the reduction of body weight and body fat, when compared to the lifestyle program or LCn-3 supplementation alone. The total body weight lost relates to a clinically significant amount of weight loss, and was shown to continue after the initial 12-week intervention. These changes were noted while LBM was consistent and unchanged for the three intervention groups.

Furthermore, LCn-3, the lifestyle program nor both combined significantly influenced measures of inflammation or quality of life. It is likely that the lack of change in inflammation was due to the overall lower baseline value of CRP in this population. Similarly, quality of life was shown to improve for all groups and time from treatment is likely to have influenced this most profoundly.

An interesting finding of the trial was the LCn-3 mediated improvement in grip strength and physical function from week 12 to 24, independent of exercise. This will need further validation from more detailed assessment, but agrees with previous literature that reported a positive effect for LCn-3 on grip strength and gait function.

Section 2 reports on potential confounding factors not fully explored in the published manuscript. These analyses indicated that there were no significant changes between groups for dietary energy and protein intake. In addition, no significant differences in LBM change were observed when assessing protein intake relative to body weight over time.

Finally, differences between the groups were not noted for markers of Lymphoedema risk or menospausal symptoms.

5.1 Manuscript #5 – Submitted for Publication

Journal of Cachexia, Sarcopenia and Muscle

Muscle Mass, Omega-3, Diet, Exercise and Lifestyle (MODEL) study: a randomized trial for women after breast cancer treatment

--Manuscript Draft--

Manuscript Number:	JCSM-D-14-00100
Full Title:	Muscle Mass, Omega-3, Diet, Exercise and Lifestyle (MODEL) study: a randomized trial for women after breast cancer treatment
Article Type:	Original Article
Corresponding Author:	Cameron Keith McDonald, MSc University of Queensland St Lucia, QLD AUSTRALIA
Corresponding Author Secondary Information:	
Corresponding Author's Institution:	University of Queensland
Corresponding Author's Secondary Institution:	
Corresponding Author E-Mail:	c.mcdonald4@uq.edu.au
First Author:	Cameron Keith McDonald, MSc
First Author Secondary Information:	
Order of Authors:	Cameron Keith McDonald, MSc
	Sandra Capra, Professor
	Judy Bauer, Associate Professor
	Earl Duncan, MSc - Statistics
Order of Authors Secondary Information:	
Abstract:	Long chain omega-3 fatty acids (LCn-3s) may enhance the effect of an anabolic stimulus on LBM. This has not been investigated in women after treatment for breast cancer who are at risk of LBM loss. Our aim was to compare change in LBM, QOL and inflammation after LCn-3 supplementation alone (N-3), a nutrition and exercise (aerobic (AET) and resistance (RET) training) lifestyle program plus olive oil (EP+OO), or the lifestyle program plus LCn-3 (EP+n-3). Forty-nine women who had completed treatment for breast cancer (48.9 + 1.4 years) were randomly assigned to 3 groups. One consumed 5g of LCn-3s (N-3; open-label); two groups participated in a 12-week exercise and nutrition lifestyle program. In a double blind fashion, one consumed 5g/d of olive oil (EP+OO), while the other had 5g/d of LCn-3s (EP+N-3). Measures of body composition, CRP, QOL and physical function were taken at baseline, 12 and 24 weeks. No change was noted in LBM or CRP after 24 weeks, QOL improved for all groups equally. EP+N-3 experienced greater reductions in body weight, waist and hip girth. Exercise prescription improved upper body strength-endurance and LCn-3 supplementation attenuated decreases in handgrip strength. LCn-3 did not augment the LBM or CRP response to a lifestyle program, however combining LCn-3 with the lifestyle program may improve markers of adiposity relevant to breast-cancer specific outcomes. LCn-3 may also enhance maintenance of grip strength over time. A larger trial using exercise and LCn-3 with a focus on cardio-metabolic and physical function outcomes is warranted.
Suggested Reviewers:	Margaret Allman-Farinelli, Associate Professor University of Sydney margaret.allman-farinelli@sydney.edu.au Good reputation in dietetics and nutrition in Australia
	Elizabeth Isenring, Associate Professor Bond University lisenrin@bond.edu.au

	Excellent reputation in cancer research
Author Comments:	This article is the first to comment on very practical measures of physical function and how they relate to body composition and metabolic factors in breast cancer populations. We believe this information to be helpful to medical and complementary practitioners in discussing lifestyle factors with breast cancer survivors.

Title Page

Title:

Muscle Mass, Omega-3, Diet, Exercise and Lifestyle (MODEL) study: a randomized trial for women after breast cancer treatment

Short running head:

`

Authors:

Cameron Keith McDonald, MSc (Nut & Diet) – c.mcdonald4@uq.edu.au - 70%

Professor Sandra Capra – s.capra@uq.edu.au - 10%

Associate Professor Judy Bauer – j.bauer1@uq.edu.au - 15%

Earl Duncan, Statistician – e.duncan@wesleyresearch.com.au - 5%

Institutions where the research was carried out

1. Centre for Dietetics Research (C-Diet-R), University of Queensland, Brisbane, Queensland, Australia (CKM, SC, JB)
2. The Wesley Research Institute, Level 8, East Wing, The Wesley Hospital, Auchenflower, Queensland, Australis

Corresponding Author

Name: Cameron McDonald;

Address: Level 5, Human movement studies building, University of Queensland, QLD, Australia, 4072

Phone: +61411380566

Email: c.mcdonald4@uq.edu.au

Reprints: reprints will not be available from the author

Abbreviations

LBM – Lean body mass; BF% - Body fat percentage; QOL: Quality of life

Trial Registration

UTN: U1111-1116-8520

ACTRN: ACTRN12610001005044

Full protocol: URL: http://www.biomedcentral.com/1471-2407/14/264

Abstract

Background

Long chain omega-3 fatty acids (LCn-3s) may enhance the effect of an anabolic stimulus on LBM. This has not been investigated in women after treatment for breast cancer who are at risk of LBM loss. Our aim was to compare change in LBM, QOL and inflammation after LCn-3 supplementation alone (N-3), a nutrition and exercise (aerobic (AET) and resistance (RET) training) lifestyle program plus olive oil (EP+OO), or the lifestyle program plus LCn-3 (EP+n-3).

Methods

Forty-nine women who had completed treatment for breast cancer (48.9 $\pm$ 1.4 years) were randomly assigned to 3 groups. One consumed 5g of LCn-3s (N-3; open-label); two groups participated in a 12-week exercise and nutrition lifestyle program. In a double blind fashion, one consumed 5g/d of olive oil (EP+OO), while the other had 5g/d of LCn-3s (EP+N-3). Measures of body composition, CRP, QOL and physical function were taken at baseline, 12 and 24 weeks.

Results

No change was noted in LBM or CRP after 24 weeks, QOL improved for all groups equally. EP+N-3 experienced greater reductions in body weight, waist and hip girth. Exercise prescription improved upper body strength-endurance and LCn-3 supplementation attenuated decreases in handgrip strength. LCn-3 did not augment the LBM or CRP response to a lifestyle program, however combining LCn-3 with the lifestyle program may improve markers of adiposity relevant to breast-cancer specific outcomes.

Conclusions

LCn-3 may also enhance maintenance of grip strength over time. A larger trial using exercise and LCn-3 with a focus on cardio-metabolic and physical function outcomes is warranted.

Key Words – Breast cancer; Nutrition; Omega-3 fatty acids; Exercise; RCT

INTRODUCTION

After treatment for breast cancer, women typically experience a decrease in lean body mass (LBM) with a concurrent increase in measures of adiposity (subcutaneous, waist, visceral) with or without body weight change.[1] These changes are associated with a higher risk of metabolic syndrome related diseases after treatment.[2] Chemotherapy, younger age, and physical inactivity have been associated with increases in adiposity.[3] In contrast, mechanisms underpinning LBM change in breast cancer survivors are not fully known. Chemotherapy may contribute to LBM loss through the associated myotoxicity.[4] while decreased physical activity and chronic inflammation have been hypothesized as potential causes of LBM loss.[4] On the other hand, prospective LBM increases have been associated with treatment with aromatase inhibitors (AIs),[3] while cross-sectional push up performance,[5] and cardiorespiratory fitness have been positively associated with greater weight and age adjusted LBM.[5]

Some,[6, 7] but not all[8] exercise interventions have reported an increase in LBM (0.7 to 1kg) compared to control; with positive results coming from aerobic (AET)[9] and resistance training (RET) alone[7] or combined.[10] No effect for exercise has been seen on total body weight, however, reductions in BF%and waist girth relevant to metabolic syndrome have been reported.[3] Dietary energy restriction alone has been shown to decrease body weight and BF%, however it may be at the expense of LBM and an added risk of sarcopenia.[11] Thus trials reporting maintenance of LBM and a reduction in adiposity[8, 12] through diet and exercise prescription present as the most effective option for cardio-metabolic risk factor reduction.

Long chain omega-3 fatty acids (LCn-3) have shown benefit in LBM maintenance for cancer populations.[13] while supplementation alone has had little effect in non-cancer populations.[14] Our recent review of the evidence has indicated a potentially synergistic relationship between RET and LCn-3.[14] Compared to controls, LCn-3 supplementation resulted in improved neuromuscular function and power development, muscle protein synthetic response in the fed state,[14] and greater gait speed in postmenopausal women.[15] In addition, LCn-3 may help to reduce adiposity,[16] and theoretically this may be enhanced by AET.[16] Considering the established anti-inflammatory properties of LCn-3s they present as a potential nutriceutical after treatment for breast cancer, yet have not been tested in this population.

The aim of this study was to compare change in LBM, QOL and inflammation after LCn-3 supplementation alone (N-3), a nutrition and exercise (AET and RET combined) lifestyle program plus olive oil (EP+OO), or the lifestyle

program plus LCn-3 (EP+n-3). We hypothesized that the additive effect of LCn-3 and the lifestyle program would be superior to both LCn-3 alone and lifestyle program alone.

MATERIALS AND METHODS

Participants were invited to participate through hospital breast cancer oncology centres, radio advertising, social media and breast cancer research registries in Brisbane, Australia. Eligible women as determined by telephone interview were those ≥18 years of age, with stage (Stage 0-IIIa) breast cancer; had successfully completed surgery, radiation and/or chemotherapy in the last 12 months; were able to perform moderate intensity physical activity, and BMI of >20 and <35kg/m.^{2} Exclusion criteria: a previous diagnosis with cardiovascular disease or diabetes; or, consumption of >1g/day of EPA and DHA LCn-3s combined. After baseline assessment, all measures were repeated at 12 and 24 weeks. Detailed rationale, study protocol for the full trial has been published previously. The study received ethical approval by the Uniting Care Health (UCH HREC #1034) and the University of Queensland (UQ HREC #2011000079) ethics committees.

The study was a prospective, three-armed, randomized controlled trial. Allocation of entry to N-3, EP+OO, or EP+N-3 was determined with the use of the NQuery Version 7 (Statistical Solutions Ltd, Ireland) mixed block design to randomise group order in a ratio 1:1:1, and applied to the capsule bottles at the point of production. The randomisation was concealed from the primary investigator and applied to supplement bottle by the manufacturer who had no contact with any participants. EP+N-3 and N-3 consumed 5g of marine-triglcyeride/d (Omega Daily, Blackmores Ltd, Australia), which provided a total of 1.75g EPA and 1.25g DHA per day. The EP+OO group consumed 5g of olive oil. All groups consumed capsules for the whole study period of 24 weeks. All capsules were identical in colour and shape, and with the exception of the open-label N-3 group, EP+OO and EP+N-3 were assigned such that participants and the primary investigator were blinded. Batch testing of the capsules indicated that the capsules contained the stated amount and proportion of LCn-3s (testing carried out by Alpha Laboratories, NZ).

Exercise and Nutrition Education Program

EP+N-3 and EP+OO groups attended 9 nutrition and exercise sessions (60-75min each) over the first 12 weeks. Exercise prescription progressed from one set of six exercises (10 to 20 reps) for 2wks, to two to three sets of nine exercises (10 to 20 reps) at 6 weeks. Home exercises (same as during session) were prescribed with the aim of achieving three RET and three >30 minute AET sessions per week. RET included push-ups, squats*, lunges, glute bridging, seated row*, shoulder press*, bicep curls* and a series of postural and abdominal exercises (*Exercises

marked indicates the use of the Gymstick™). The Gymstick™, a specialised elastic resistance stick, which has been used in a previous non-cancer population of similarly aged participants was used for some of the exercises.[17] AET was prescribed at an RPE level of 11 to 13 for weeks one to three, and then 12 to 14 for the remainder of the intervention. Participants were able to select their preferred mode of AET. A handbook and narrated powerpoint slides were made available to those in the lifestyle program for reviewing at home. Attendance was noted for each session, and exercise diaries were completed by the women to capture weekly exercise completed. The Primary Investigator who was an Accredited Practising Dietitian and Accredited Exercise physiologist with relevant clinical experience facilitated the sessions. After 12 weeks, EP+OO and EP+N-3 participants were encouraged to continue the exercise program; all three groups recorded their exercise during this time. The N-3 group were encouraged to exercise and eat as they wished, and were offered the program after the intervention.

Side effects of treatment

All participants were asked to report the appearance of any adverse symptoms that may be related to the exercise program or capsule consumption. In cases of both exacerbation of lymphedema or severe gastro-intestinal upset, exercises and supplements were ceased, respectively, until symptoms had abated and cause determined by a medical professional.

Anthropometric Variables – Primary outcome

Height without shoes was measured to the nearest 0.5cm using a stadiometer (Seca). Weight to the nearest 0.1kg, LBM and fat mass were measured using the BODPOD digital scales and air displacement plethysmography (ADP) (BODPOD, COSMED USA Inc), respectively. Before each assessment day, the BODPOD scales and air chamber were calibrated as per the manufacturer's instructions using known weights and volumes. All measures were performed by a certified BODPOD assessor. Results were expressed as percentage LBM and body fat of total weight, from which absolute LBM (kg) was then calculated.

Quality of Life (QOL) – Secondary outcome

QOL was measured using the Functional Assessment of Cancer Therapy- Breast + 4 (FACT-B+4)tool completed at baseline, 12wk and 24wk time-points.[18] Fatigue was measured using the additional 13-item FACT-F tool.[19]

Blood analyses – Secondary outcome

Inflammation

Fasting high sensitivity-C Reactive Protein (CRP) was measured using a latex-enhanced immunoturbidimetric assay of blood serum. The 8.5ml sample of fasting whole blood was collected and analysed for CRP on the day of collection.

Fatty acid testing

The sample was frozen at -20^{o}C, and transported to Healthscope Pathology, Victoria. Lipids from red cells were extracted with chloroform methanol mixture. The fatty acids were trans-esterificated to methyl esters with methylation reagent "Meth-Prep 2". The methylation extract was then analysed by gas liquid chromatography method with flame ionisation detection (gas chromatograph Schimadzu G-2010-FID). The proportion of fatty acids content of the erythrocytes expressed as % of total fatty acids.

Pill count

Participants were asked to return all bottles, both empty and partially consumed and a pill count was carried out at the 12 and 24 week time points.

Muscle function and fitness tests

Upper body strength-endurance was determined by performing the maximum number of push-ups (knees on ground) in 1 minute.[20] Lower body muscular endurance was measured using a 1-minute sit-stand test. Chair height was standardised at 43cm[20]. Hand grip strength was performed on both arms, with the maximum of 3 attempts recorded.

Sub-maximal aerobic capacity was measured using the modified Balke sub-maximal treadmill test. Seated blood pressure was measured before each assessment to ensure safety of exercise. The test being completed when the individual had reached 85% of their estimated maximum heart rate (max HR) (est. maxHR = 220-age). The Health Assessment Questionnaire-Disease Index (HAQ-DI) was completed to determine physical functional ability represented by the sum of scores generated from the eight subscales. A lower score indicates greater functional ability.

Physical activity

Daily physical activity was objectively measured using GT1M accelerometers (Actigraph, USA). Participants were asked to wear the device for at least 10 hours per day for one week at each time point. Activity counts were divided into % time spent in: sedendary, light and $\geq$ moderate intensity activity. The participants also completed the Active Australia Survey[21], and EP+N-3 and EP+OO recorded all prescribed exercises performed throughout the 24 week period in a study-specific exercise diary.

Diet history

Dietary intake was measured by the practitioner assisted Diet History Questionnaire.[22] The primary investigator reviewed the questionnaire with the participant to clarify portion sizes and ensure completion of the form. Nutrient intake analysis was carried out using Foodworks 7 (Xyris Software) and converted to daily intake.

Lymphoedema Index

Extra-cellular fluid in the upper limb was measured by BIS L-dex XCA™ (Bio-Impedimed, Queensland).[23] Electrodes were placed at anatomical landmarks at the wrist of each arm and right ankle by a trained research assistant. An increase in extra-cellular fluid is paralleled by a decrease in impedance and the result recorded as a ratio to the non-affected arm, taking into consideration arm dominance.[24]

Statistical analysis and sample size calculation

Differences in demographic data between groups were assessed by ANOVA and Kruskal Wallis tests, for parametric and non-parametric data, respectively. The Friedman test and repeated measures ANOVA were used to determine within group differences over 3 time points, and Wilcoxon Signed Rank tests and paired t-tests with Bonferroni adjustment for multiple comparisons were used to determine change within-groups between two time points. The effects of treatment on the dependent measures were analysed by a 3 x 3 factorial repeated measures ANOVA with group treatment (N-3 vs EP+OO vs EP+N-3), exercise treatment (EP+OO & EP+N-3 or N-3), LCn-3 treatment (N-3 & EP+N-3 or EP+OO) and additive treatment (EP+N-3 or EP+OO & N-3). Intention-to-treat was used for all analyses. To optimise the analysis of differences between treatments, when appropriate, a nested ANOVA design was used to examine changes in dependent variables from baseline nested in time. The primary outcome measure is change in lean body mass (LBM) at 24 weeks. A mean change in lean body mass over 12 weeks of 0.8kg[9, 25] has been observed in group previous exercise and nutrition trials in breast cancer populations. Assuming that the minimum difference in LBM across the comparison groups is a mean of 1kg, 38 participants per group will be required to detect this difference with 90% power and type 1 error of 5% or less (two-tailed). A total of 114 participants was therefore required. Assuming 10% for attrition and allowing 15% for contingency, 51 subjects per group will need to be recruited to obtain complete data on at least 38 for each group

Results

Participants were recruited over a 15-month period (Oct 2011 – Jan 2013) (Fig 1). A total of 135 women were initially screened for inclusion criteria, recruitment was stopped due to time constraints. The major reasons for

exclusion were >12 months post treatment completion, and daily consumption of >1000mg EPA and DHA combined. Forty-nine participants were eligible for the study and completed baseline assessment. Forty-three (87.8%) participants returned at 12-wks, and 42 (85.7%) at 24-wks time-points. Forty-nine participants were included in the primary analyses. Descriptive statistics of the population are shown in Table 1. No significant differences were found between group for any demographic, treatment or body composition parameters.

The EP+OO and EP+N-3 groups attended a mean of 7.06±2.4 and 6.6±2.09 out of 9 sessions, respectively. Of the participants allocated to EP+OO and EP+N-3 groups that submitted their exercise log (70%), they performed 67% and 72.3% of the prescribed RET, and 130.2% and 117.3% of the prescribed AET volume. In addition, from 12 to 24 week time points, all three groups performed equal amounts of physical activity as measured by exercise diary and accelerometry (data not shown). One participant was excluded from CRP analyses at 12wks as her CRP was measured as 26.2mg/L, which was taken during an upper respiratory tract infection

LCn-3 concentration within erythrocytes (RBC)

Total n-3 fatty acids increased by 65%, 10% and 88% for N-3, EP+OO and EP+N-3, respectively. For N-3 and EP+N-3, 80% of the change was due to an equal 40% increase in both EPA and DHA (Figure 2), which resulted in a significant LCn-3 x time interaction for total n-3 ($p=0.018$), EPA ($p<0.000$), but not DHA ($p=0.113$). EP+N-3 experienced a significantly greater increase in DHA at 24 weeks than EP+OO (+1.9% vs. +0.54%, $p=0.023$, respectively); N-3 displayed a within-group trend for increased DHA, but not compared to EP+OO (change at 24 weeks: +1.54%; within: $p=0.076$, between: $p=0.979$). EP+OO did not experience any significant increases in EPA, DPA or DHA at 12 or 24 weeks ($p<0.05$).

Accounting for all those who returned all capsule bottles (n=41), N-3, EP+OO and EP+N-3 consumed 84.6±21.6%, 81.8±33.4% and 83.4±24.6% of the allocated capsules. Controlling for baseline levels of LCn-3s, capsule consumption tended to be correlated with erythrocyte levels of EPA, with no significant correlation seen for concentration of DHA.

Changes in measures of body composition

A significant treatment x time was found for change in LBM ($F=2.6$, $p=0.04$) Figure 3. Pairwise comparisons revealed that after 12 weeks, EP+OO had a significantly greater increase in LBM than N-3 (+0.6kg±1.4 vs. -0.64kg±1.3; $F=7.3$, $p=0.011$. After 24 weeks, differences in change from baseline disappeared (N-3: -0.13kg±1.4 vs. EP+OO: +0.42kg±1.5; $F=2.78$, $p=0.106$). Change in LBM for EP+N-3 (12wk: -0.07kg±0.8 & 24wk: -0.38kg±1.3)

was not significantly different to either N-3 (F=2.65, p=0.113, respectively) or EP+OO (F=2.83, p=0.103, respectively) at either time point. A significant LCn-3 x time interaction was found, which indicated that EP+OO experienced greater gains in LBM after 12 weeks compared to those consuming LCn-3 (-0.34kg±1.0 vs. +0.6kg±1.4, respectively; F=7.0, p=0.011). However at 24 weeks, there was no difference between the groups (-0.09kg±1.4 vs. +0.42kg±1.5, respectively, F=2.5, p=0.115).

For total body weight, only the EP+N-3 group experienced a significant decrease at 12 and 24 wks (change from baseline: -1.3±1.3kg and -2.3±1.9kg, respectively). No statistically significant change was noted for EP+OO (-0.36±1.6kg and -0.86±2.4kg) or N-3 (-.3±2.5kg and -0.83±2.0kg). No significant time x treatment interaction was observed for body weight, BF%, waist girth or hip girth (Figure 4A-D). Significant additive x time interactions were observed for body weight (p=0.042), while waist (p=0.143) and hip (p=0.07) approached statistical significance. No effect was observed for body fat% (p=0.509). Nested analyses indicated that compared to N-3 & EP+OO combined, the EP+N-3 (additive) group experienced a significantly greater reduction in body weight (-0.84kg±2.9 vs. -2.27kg±1.9, p=0.028) and hip girth (-0.87cm±2.33 vs. -2.26cm±1.8, p=0.038) at 24 weeks. In addition, compared to N-3 & EP+OO, EP+N-3 had a greater reduction in waist girth at 12 weeks (-0.23cm±1.9 vs. -1.4cm±1.8, p=0.045), which was clinically meaningful, yet statistically non-significant at 24 weeks (-0.84cm±2.9 vs. -2.3cm±2.5; F=3.028, p=0.088).

Change in CRP

No significant interaction for time x treatment was noted for CRP at 12 or 24 week time points (p=0.319) Table 2. No significant exercise x time, LCn-3 x time or additive x time interactions were found (P>0.05). Pairwise comparisons indicated that EP+OO experienced a greater decrease in CRP than EP+N-3 at 12 weeks (-0.65±1.5 vs. +0.24±0.65, p=0.036), which was attenuated at 24 weeks (-0.77±1.5 vs. +0.1±1.0, p=0.07). No other significant differences were reported at either time point. Within group analyses indicated that both N-3 and EP+OO groups experienced non-significant decreases in CRP from baseline to 24 weeks (N-3: -0.39, p=0.133; EP+OO: -0.77, p=0.081), while the EP+N-3 experienced no change (+0.1, p=0.698).

Change in measures of quality of life and fatigue

No significant time x treatment interaction was found for FACT-B+4 (p=0.745) Table 2. Nor were there any significant exercise x time, LCn-3 x time or additive x time interactions (p>0.05). Within group analyses indicated that only EP+OO experienced a significant increase in FACT-B+4 after 12 weeks (+6.4±9.5, p=0.049), while changes for EP+N-3 (+6.8±12.0, p=0.094) and N-3 groups (+5.6±8.9, p=0.072) did not reach statistical significance. Numerical, but non-significant increases in FACT-B+4 score were seen for all groups. However, a main effect for

time was found corresponding with an increase in FACT-B+4 for all groups combined at 12 and 24wks (p=0.000 for both).

No significant interactions were noted for FACT-F. In addition, no significant change was noted within groups at either time

Changes in parameters of physical function

All groups improved in number of push-ups performed over time (Table 3). A significant exercise x time interaction was found (p=0.004); compared to N-3, those who participated in the lifestyle program experienced greater improvement after 12 weeks (change in push ups performed: 0±11.1 vs. 'exercise' = 8.3±7.6, p=0.003). From 12 to 24 weeks, both groups (N-3 and lifestyle program) experienced similar improvement (4.1±10.8 and 1.49±5.3, respectively, p=0.244), yet change from baseline remained significantly different at 24wk (N-3: 4.18±5.3 and lifestyle group: 9.8±8.7; p=0.022).

No significant time x treatment interaction was found for squats performed (p=0.766), which was maintained when exercise groups were combined (F=0.939, p=0.398). There was a significant main effect for time (p=0.000). Within groups, compared to baseline, all 3 treatment groups significantly improved the number of squats performed ($p<0.05$) (Table 3).

No significant time x treatment interaction, or main effect for time was found for time to 85% predicted HR in the sub-max treadmill test (p=0.267 & p=0.555, respectively).

No significant time x interaction was found for handgrip strength (F=1.9, p=0.1) (Table 3). No exercise x time, LCn-3 x time, or additive x time interactions were indicated at 12 weeks (F=0.54, p=0.588). However, while no difference was noted at 12wks ($p<0.05$), from 12 to 24wks a significant LCn-3 x time interaction was observed (p=0.013). This indicated a maintenance in grip strength for the LCn-3 supplemented groups compared to decrease in strength for the EP+OO group (0.05kg±0.37 and -0.94kg±2.12, respectively). These results were similar regardless of whether dominant or non-dominant, or treatment affected or unaffected arm were used.

A non-significant treatment x time interaction was observed for HAQ-DI scores (p=0.092). The Ex+N-3 group experienced a significant decrease from baseline to 24 wks (-1.67±0.69, p=0.035), while no significant reductions were seen at 24 wks for the LCn-3 (-0.57, p=0.422) or Ex+OO (-0.067±0.6, p=0.809). Pairwise comparisons revealed no differences in change from baseline to 12 wks ($p>0.05$). However, a significant LCn-3 x time interaction from 12wks to 24wks (p=0.046) was observed (Figure 3). This indicated that those who consumed LCn-3 experienced greater improvements in physical function compared to those who did not consume LCn-3 (Mean

change: LCn-3 group= -0.83±1.62 vs 0.26±1.75). No significant hormonal therapy x time interaction was observed, which indicated there was no between-group effect of hormonal treatment on change in HAQ-DI.

Covariates for LBM and body composition change

No differences were noted between any group at either time point for overall energy and protein intake, nor for objective (accelerometry and treadmill test) or subjective physical activity measures (data not shown).

Discussion

The combination of LCn-3 and an exercise and nutrition lifestyle program has no advantage over either intervention alone for change in LBM, QOL or inflammation. However, greater reductions in body weight and measures of adiposity were observed in those exposed to the lifestyle program and LCn-3 supplementation. The magnitude of body weight and waist decrease was clinically significant to post-treatment mortality and morbidity. All groups experienced equal and significant improvements in QOL.

Our observations of LBM are similar to previous interventions combining exercise and nutrition.[8] However, longer and supervised trials have reported LBM gain over 6 to 12 months of 0.7kg to 1kg.[7] The EP+OO group experienced a significantly greater increase in LBM at 12wks; however, this was not maintained at 24 weeks. The exercise diaries indicate lifestyle program participants achieved ~70% adherence to prescribed RET while ~130% of prescribed AET; this shift to AET with the increased activity seen in the N-3 group may explain the lack of difference between groups, and no absolute gains in LBM and muscle strength generally.

Apart from LBM, moderate total body weight loss is becoming a target to reduce all-cause mortality ,with a 2.7kg been shown to improve survival over 6 years of follow up.[2] Thus, the combination of LCn-3 and the lifestyle program elicited a clinically significant and greater reduction in waist girth at 12 weeks and body weight and hip girth at 24 weeks compared to the other two groups. This finding is of considerable relevance to both health practitioners and individuals. Body image due to the muscle and fat changes after treatment is a psychological stressor for survivors, and reduction of visceral fat (waist circumference) is of benefit to mortality and morbidity. Previous trials using LCn-3 with or without energy restriction have shown reductions in total fat mass and adipocyte diameter[16] however AET and LCn-3 in combination has not elicited greater reductions in fat mass previously.[26] In vitro and animal studies have repeatedly indicated an up regulation of fatty acid oxidation due to alterations in fatty acid transport to and metabolism within the mitochondria, reviewed in detail here[16]. AET is known to

similarly enhance mitochondrial density and enzyme concentration, thus this research provides a theoretical basis to explain the greater body weight and adiposity reduction observed in our study.

Consumption of LCn-3 supplements resulted in an increase in erythrocyte concentration of EPA and DHA when compared to olive oil, however both N-3 did not experience statistically significant increases over EP+OO. We did notice an accumulation effect where %DHA content was significant at 24 weeks but not 12, which is likely to be due to the gradual incorporation of DHA into cells over time.[5] However, pill counts reflected equal adherence between the three groups.

Compared to lifestyle plus LCn-3, lifestyle program plus olive oil experienced a greater decrease in CRP at 12 weeks, but this disappeared at 24 weeks. Our results match findings from previous exercise trials studies that reported no change in CRP after exercise in breast cancer[27] and healthy populations. Greater concentration of CRP has been associated with cross-sectional weight in our group at baseline[5] and other studies, however, changes in weight and CRP have not been shown to correlate in breast cancer and other populations.[12]

Our data indicate that all interventions equally improved QOL in this breast cancer population. Previous exercise and/or nutrition interventions with a measure of quality of life have reported mixed findings. Overall quality of life,[28] physical function,[6] and psychosocial[28] subscales have been shown to improve after exercise only interventions. In contrast, similar to our study previous exercise and diet combined trials have not shown significant between group improvements.[8, 12] To explain this, exercise diaries indicated that the N-3 group performed as much AET as those in the lifestyle program from 12 and 24wk assessments. Previously, increased quality of life scores have been associated with greater aerobic fitness, and for all survivors, QOL has been observed to improve over time without intervention.[29] Thus we are unable to disentangle the effects of LCn-3, lifestyle program participation, or time since the end of treatment. However, it is a positive result that the population as a whole improved regardless of the intervention type.

It was expected that the lifestyle program would elicit an increase in muscle strength, however no differences were found between the groups for lower body strength-endurance. While there was an effect for the lifestyle group and push up performance, the lack of difference between the olive oil and LCn-3 groups indicates LCn-3 had no influence on strength development over time. Participation in aerobic exercise was higher than prescribed in the intervention for all groups, however this was not reflected in fitness levels, as there was no change noted for any group in the treadmill test. Accelerometer data also indicated no change in moderate/vigorous physical activity. A few reasons may explain this disparity. Firstly, it is possible that participants over-calculated their weekly physical

activity; accelerometers were needed to be removed for water based activities, thus would have not accounted for swimming of which was common; or the aerobic activity may not have been performed at a high enough intensity to improve VO_2max. Prior research has indicated that an intervention utilizing supervised and objectively measured exercise may be the most effective in augmenting body composition and fitness levels, which may partly explain our results.[9]

Adherence to resistance training of ~70% may have been insufficient to promote significant LBM or strength change. Verbal feedback from participants indicated that motivation to perform the resistance training was the major factor for the adherence noted. Previous studies that have employed more frequent supervision in gym-based programs have recorded significantly greater increases in strength,[7, 30] thus the potential for home based elastic-resistance exercises may only be sufficient to maintain LBM.

Both HAQ-DI and handgrip strength were enhanced and better maintained for those consuming LCn-3 independent of exercise. Previously, compared to lower body RET alone, Rodacki et al (2012) observed a significant improvement after LCn-3 supplementation plus RET in muscle power generation via improved electromechanical delay in middle aged women.[31] Since our participants were tested for muscle strength-endurance, improved neuromuscular function may not have provided any advantage to LCn-3 supplemented individuals. However, handgrip strength and physical function (measured by HAQ-DI) was maintained in the LCn-3 supplemented groups, while lifestyle plus olive oil group experienced a decrease in both parameters from 12 to 24 weeks. It is plausible that similar to Rodacki et al,[31] LCn-3 may have improved neuromuscular activation leading to a better maintenance of force generation in the LCn-3 groups. Furthering this, Robinson et al previously reported that every additional serve of fatty fish was associated with 0.43kg and 0.48kg increase in handgrip strength for older (59 to 73 years) men and women, respectively.[32] Additionally, Murphy et al, found that compared to a standard care control, the LCn-3 group experienced significantly lower chemotherapy induced infiltration of intra-muscular triglyceride (IMTG).[13] Where greater IMTG was related to a lower plasma concentration of EPA,[13] and previously has been associated with poorer physical function and strength in older populations.[33] It is acknowledged that the magnitude of change in handgrip strength is not large, such that a longer duration of follow up would be required to confirm the trends noted. With regard to physical function measured by the HAQ-DI, one previous study by Hutchins-Wiese et al[15] reported a significant improvement in gait speed after LCn-3 supplementation, however HAQ-DI was not measured in this trial limiting a direct comparison. In regards to hormone treatment derived joint pain, we did not observe any differences in physical function between AIs and other hormonal treatments. In addition, HAQ-DI scores were not related to erythrocyte LCn-3 or HS-CRP levels.

However while oestrogen is associated with pro-inflammatory cytokine levels, these have not been consistently shown to affect AI-joint related pain.[34]

Caution should be taken in interpretation of the results as our study was limited by a small sample size due to recruitment difficulties. Of those participants who were eligible there was good uptake into the trial, thus the narrowness of our selection criteria is likely to be the critical element of our reduced sample. We calculated that 135 participants would be required in order to achieve sufficient power. In addition, to better determine the effects of LCn-3 on LBM and function, higher intensity of RET is recommended, as the previous studies indicating an effect of LCn-3 on muscle strength-power have used weights of greater specificity to LBM gains (machine and free weights).[7] On the other hand, our results are similar to those with studies that included a larger population with equivalent intervention protocols.[8, 12] Finally, our attrition rate of 16% (41/49) reflects that the intervention was well tolerated and similar to other lifestyle interventions in this population.[7, 30]

The present trial is the first to evaluate the combined effect of LCn-3 supplementation and a lifestyle program compared to either intervention alone on body composition, inflammation and quality of life outcomes in a breast cancer survivor population. No significant effect on LBM or CRP was observed for any intervention group, while all groups experienced an improvement in QOL. The additive effect of LCn-3s and the lifestyle program was related to clinically relevant and greater reductions in body weight and adiposity compared to either intervention alone. Those consuming LCn-3 supplements experienced better maintenance of grip strength and improvement in physical function compared to those consuming olive oil. Caution in the interpretation of these results is needed due to sample size. Further research involving a larger population assessing long-term cardio-metabolic and functional capacity outcomes is recommended.

Acknowledgements

The authors would like to acknowledge the support of the Wesley Research Institute for the provision of administration support *gratus*. To Dr. Michelle Palmer, PhD and Prof. David Jenkins for their ongoing review of the study and it's management.

The authors of this manuscript certify that they comply with the ethical guidelines for authorship and publishing in the Journal of Cachexia, Sarcopenia, and Muscle 2010;1:7–8 (von Haehling S, Morley JE, Coats AJ and Anker SD).

Conflicts of interest/funding support:

Blackmores Pty LTd supplied all fish oil and olive oil capsules *gratis*. Their involvement was excluded from all data collection, analysis and manuscript development. The Wesley Research Institute provided funding for blood analyses, accelerometers, body composition assessment by research assistant, recruitment costs and other trial related matters. Australian Postgraduate Awards provided funding for CM as part of their PhD candidature. Fish oil supplements and olive oil capsules were provided by Blackmores Pty Ltd gratus. They were not involved in any aspect of the trial, including dose selection.

The Wesley Research Institute provided funding for all blood analyses, paid research assistant hours and exercise apparatus for all participants. No conflict of interest existed for this body.

References

1. McDonald, C.K., J.D. Bauer, and S. Capra, *Body Composition and Breast Cancer- the Role of Lean Body Mass.* Cancer Forum, 2011. **35**(2): p. 102-106.
2. Ligibel, J.A. and P.J. Goodwin, *NEW and RENEW: Building the Case for Weight Loss in Breast Cancer.* Journal of Clinical Oncology, 2012. **30**(19): p. 2294-2296.
3. Sheean, P.M., K. Hoskins, and M. Stolley, *Body composition changes in females treated for breast cancer: a review of the evidence.* Breast Cancer Research and Treatment, 2012. **135**(3): p. 663-680.
4. Mourtzakis, M. and M. Bedbrook, *Muscle atrophy in cancer: a role for nutrition and exercise.* Applied Physiology, Nutrition and Metabolism, 2009. **34**(5): p. 950-956.
5. McDonald, C., *Muscle function and omega-3 as predictors of lean body mass in women after breast cancer treatment.* SpringerPlus, 2014.
6. Courneya, K.S., et al., *Effects of Aerobic and Resistance Exercise in Breast Cancer Patients Receiving Adjuvant Chemotherapy: A Multicenter Randomized Controlled Trial.* J Clin Oncol, 2007. **25**(28): p. 4396-4404.
7. Schmitz, K.H., et al., *Safety and Efficacy of Weight Training in Recent Breast Cancer Survivors to Alter Body Composition, Insulin, and Insulin-Like Growth Factor Axis Proteins.* Cancer Epidemiology, Biomarkers & Prevention, 2005. **14**(7): p. 1672-1680.
8. Demark-Wahnefried, W., et al., *Results of a Diet/Exercise Feasibility Trial to Prevent Adverse Body Composition Change in Breast Cancer Patients on Adjuvant Chemotherapy.* Clinical Breast Cancer, 2008. **8**(1): p. 70-79.
9. Irwin, M.L., et al., *Exercise Improves Body Fat, Lean Mass, and Bone Mass in Breast Cancer Survivors.* Obesity, 2009. **17**(8): p. 1534-1541.
10. Fernández-Lao, C., et al., *Water versus land-based multimodal exercise program effects on body composition in breast cancer survivors: a controlled clinical trial.* Supportive Care in Cancer, 2013. **21**(2): p. 521-530.
11. Thomson, C.A., et al., *Changes in Body Weight and Metabolic Indexes in Overweight Breast Cancer Survivors Enrolled in a Randomized Trial of Low-Fat vs. Reduced Carbohydrate Diets* Nutrition and Cancer, 2010. **62**(9): p. 1142-1152.
12. Djuric, Z., *A Diet and Exercise Intervention during Chemotherapy for Breast Cancer.* The Open Obesity Journal, 2011. **3**(1): p. 87-97.
13. Murphy, R.A., et al., *Nutritional intervention with fish oil provides a benefit over standard of care for weight and skeletal muscle mass in patients with nonsmall cell lung cancer receiving chemotherapy.* Cancer, 2011. **117**(8): p. 1775-1782.
14. McDonald, C.K., J.D. Bauer, and S. Capra, *Omega-3 fatty acids and changes in LBM – alone or in synergy for better muscle health? A Review.* Canadian Journal of Physiology and Pharmacology, in press, 2013.
15. Hutchins-Wiese, H.L., et al., *The impact of supplemental n-3 long chain polyunsaturated fatty acids and dietary antioxidants on physical performance in postmenopausal women.* The journal of nutrition, health & aging, 2013. **17**(1): p. 76-80.
16. Buckley, J.D. and P.R.C. Howe, *Long-Chain Omega-3 Polyunsaturated Fatty Acids May Be Beneficial for Reducing Obesity—A Review.* Nutrients, 2010. **2**(12): p. 1212-1230.
17. Lubans, D.R., et al., *Pilot randomized controlled trial: elastic-resistance-training and lifestyle-activity intervention for sedentary older adults.* Journal of aging and physical activity, 2013. **21**(1): p. 20.

18. Cella, D., et al., *The Functional Assessment of Cancer Therapy scale: Development and validation of the general measure.* J Clin Oncol, 1993. **11**(3): p. 570 - 579.
19. Cella, D., et al., *Combining Anchor and Distribution-Based Methods to Derive Minimal Clinically Important Differences on the Functional Assessment of Cancer Therapy (FACT) Anemia and Fatigue Scales.* Journal of Pain and Symptom Management, 2002. **24**(6): p. 547-561.
20. American College of Sports Medicine, ed. *ACSM's Guidelines for Exercise Testing and Prescription 8th Ed*. 2010, Wolters Kluwer/Lippincott Williams & Wilkins: Philadelphia.
21. Brown, W.J., et al., *Reliability and validity of a modified self-administered version of the Active Australia physical activity survey in a sample of mid-age women.* Australian and New Zealand Journal of Public Health, 2008. **32**(6): p. 535-541.
22. Martin, G.S., *The interviewer-administered, open-ended diet history method for assessing usual dietary intakes in clinical research: relative and criterion validation studies*, in *University of Wollongong Thesis Collection*. 2004, University of Wollongong: Wollongong.
23. Czerniec, S.A., et al., *Assessment of breast cancer-related arm lymphedema-comparison of physical measurement methods and self-report.* Cancer Investigation, 2010. **28**: p. 54-62.
24. Rockson, S.G., *Bioimpedance analysis in the assessment of lymphoedema diagnosis and management.* Journal of Lymphoedema, 2007. **2**: p. 44-48.
25. Herrero, F., et al., *Combined Aerobic and Resistance Training in Breast Cancer Survivors: A Randomized, Controlled Pilot Trial.* International Journal of Sports Medicine, 2006. **27**(7): p. 573-580.
26. Tiryaki-Sönmez, G., B. Schoenfeld, and S. Vatansever-Ozen, *Omega-3 fatty acids and exercise: a review of their combined effects on body composition and physical performance.* Biomedical Human Kinetics, 2011. **3**: p. 23-29.
27. Jones, S.B., et al., *Effect of Exercise on Markers of Inflammation in Breast Cancer Survivors: The Yale Exercise and Survivorship Study.* Cancer Prevention Research, 2013. **6**(2): p. 109-118.
28. Ohira, T., et al., *Effects of weight training on quality of life in recent breast cancer survivors.* Cancer, 2006. **106**(9): p. 2076-2083.
29. Hsu, T., et al., *Quality of Life in Long-Term Breast Cancer Survivors.* Journal of Clinical Oncology, 2013. **31**(28): p. 3540-3548.
30. Schmitz, K.H., et al., *Weight Lifting in Women with Breast-Cancer-Related Lymphedema.* the New England Journal of Medicine, 2009. **361**(7): p. 664.
31. Rodacki, C.L., et al., *Fish-oil supplementation enhances the effects of strength training in elderly women.* The American Journal of Clinical Nutrition, 2012. **95**(2): p. 428-436.
32. Robinson, S.M., et al., *Diet and its relationship with grip strength in community-dwelling older men and women: the Hertford- shire cohort study.* J Am Geriatr Soc, 2008. **56**: p. 84-90.
33. Visser, M., et al., *Muscle mass, muscle strength, and muscle fat infiltration as predictors of incident mobility limitations in well-functioning older persons.* The journals of gerontology. Series A, Biological sciences and medical sciences, 2005. **60**(3): p. 324-333.
34. Din, O., et al., *Aromatase inhibitor-induced arthralgia in early breast cancer: what do we know and how can we find out more?* Breast Cancer Research and Treatment, 2010. **120**(3): p. 525-538.

505

Table 1. Demographical parameters of participants at baseline									
	Overall (n=49)		N-3 (n=16)		EP+OO (n=16)		EP+N-3 (n=17)		
Variable									P-value
Age (mean;SD)	48.9 (1.39)		50 (11.4)		48.9 (7.99)		47.12 (9.16)		0.687
Body Weight (kg)	73.1 (2.03)		76.4 (16.2)		73.4 (14.4)		69.7 (10.1)		0.387
BMI (Median; SD)	26.3 ()		27.9 (4.9)		26.2 (4.3)		26.1 (2.5)		0.806*
LBM (kg) Mean;SD	43.6 (0.8)		44.9 (6.9)		44.0 (4.9)		41.9 (4.7)		0.285
BF%	29.7 (1.46)		40.4 (5.8)		38.8 (8.7)		39.3 (6.2)		0.828
Days since Dx (Med;Range)	344 (150 - 961		364 (155 – 120)		338 (161-961)		295 (150-576)		0.958*
Days Since Rx Finish (Med; Range)	130 (17 – 385)		210 (32 – 203)		129.5 (37-385)		130 (17-381)		0.985
	No.	%	No.	%	No.	%	No.	%	
Postmenopausal	23	46.9	9	56.3	7	43.8	7	41.2	0.81.3
Disease stage									
I	13	26.5	5	31.3	4	25	4	23.5	
IIa	19	38.8	7	43.8	4	25	8	47.1	0.514**
IIb	9	18.4	1	6.3	4	25	4	23.5	
IIIa	8	16.3	3	18.8	4	25	1	5.9	
Surgical protocol									
Breast conservation	23	46.9	6	37.5	7	43.2	10	58.8	0.693**
Mastectomy	25	51.1	9	46.3	9	56.3	7	41.2	
Unknown	1	2	1	6.3	0	0	0	0	
Adjunctive Rx									
Chemotherapy	41	83.7	13	81.3	14	87.5	14	82.4	0.877**
Radiotherapy	33	67	8	50	12	75	13	76.4	0.298**
Taxane	37	52.9	3	18.8	11	68.8	3	17.6	0.666**
Tumour type									
HER2+	12	24.5	4	25	6	37.5	2	11.8	0.550**
Estrogen +	39	83	10	71.4	13	81.3	16	94.1	0.736**
Hormone therapy									
Tamoxifen	13	26.5	2	12.5	4	25	7	41.2	0.351**
AI	20	40.8	7	43.8	8	50	5	29.4	
None	16	32.7	7	43.8	4	25	5	29.4	
CRP (n=46) Median; Range	0.8 (0.1-10.1)		1.45 (0.1-10.1)		1.9 (0.1-8.5)		0.7 (0.1-4.3)		0.523*
% RBC Fatty Acids (n=43; Median; Range)									
EPA	1 (0.3-3.0)		0.8 (0.5-3.0)		1.15 (0.3-1.9)		0.9 (0.6-1.5)		0.496*
DHA	2.9 (0.5-5.1)		2.7 (0.7-4.2)		2.95 (0.5-5.1)		2.95 (1.6-3.6)		0.736*

N-3: Fish oil alone; EP+OO: Lifestyle program plus olive oil; EP+N-3: Fish oil plus lifestyle program. BMI: Body mass index; LBM: Lean body mass; Dx: Diagnosis; Rx: Treatment; AI: Aromatase Inhibitors; CRP: C-Reactive protein; %RBC: % of erythrocyte fatty acid composition; EPA: Eicosapentanoic acid; DHA: Docosahexanoic acid. *Kruskal Wallis test **Pearson's Chi-Square test used

Table 2. Change in Hs-CRP and FACT-B+4 scores at 12 and 24 weeks

	Bline		12wk		24wk		p-value*	p-value**
CRP (mg/L)	Mean	SE	Mean	SE	Mean	SE		
N-3	1.96	0.72	1.87	0.53	1.57	0.56	0.319	0.099
EP+OO	1.94	0.65	1.29[a]	0.54	1.47	0.37		
EP+N-3	1.22	0.32	1.47[a]	0.38	1.32	0.38		
FACT-B+4								
N-3	107.4	3.7	113	3.9	115	3.5	0.745	0.000
EP+OO	108	2.8	114.7[b]	3.4	112.1	3.6		
EP+N-3	107.7	4.9	114.5	4.1	113.7	3.7		

N-3: Fish oil only; EP+OO: Placebo plus exercise and nutrition intervention; EP+N-3: Fish oil plus exercise and nutrition intervention. CRP: C-Reactive protein; FACT-B+4: Functional Assessment of Cancer Therapy – Breast + 4 items (quality of life). No significant interaction was seen for treatment x time; no significant difference was noted with groups for change in LBM.
[a]significantly greater reduction from baseline for EP+OO, $p<0.05$
[b]significant within group increase from baseline, $p=0<0.05$;
*interaction for treatment x time at 24 weeks;
**effect for time at 24 weeks (all groups combined)

Table 3. Data for measures of physical function at 12 and 24wks

Measure	Baseline		12 wks		24wks	
	Mean	SE	Mean	SE	Mean	SE
Push Ups						
N-3	7.5	2.6	7.5[a,b]	2.1	11.9[a,b]	2.8
EP+OO	11.4	2.7	20.8[a]	3.6	20.8[a]	3.5
EP+N-3	8.5	2.5	15.8[b]	3.5	18.7[b]	3.4
Squats						
N-3	31.6	3.8	37.1	4.4	39	4.6
EP+OO	33.1	2	41.8	3.1	41.5	3.5
EP+N-3	36.3	2	42.9	2.6	43.6	2.7
Handgrip						
N-3	29.8	1.8	32.1	1.7	32.1	1.7
EP+OO	29.5	1.3	31.2	1.2	30.3	1.3
EP+N-3	29.2	1.1	30.4	1.1	30.4	1.1
Stage TMill						
N-3	10.9	0.6	12.1	0.5	12	0.4
EP+OO	12.1	0.5	12.2	0.5	11.2	0.9
EP+N-3	11.9	0.5	12.2	0.4	12.5	0.5

N-3: Fish oil only; EP+OO: Olive oil plus lifestyle program; EP+N-3: Fish oil plus lifestyle program. Push ups: number performed in 1-minute; Squats: number performed in 1-minute; Handgrip: Maximum value with dominant hand from 3 trials. A significant lifestyle program x time interaction indicated that those who participated in the lifestyle program had greater increases in push ups at 12 and 24 wks compared to fish oil only ($p<0.05$ for both). A significant oil x time interaction indicated that those who consumed fish oil maintained handgrip strength from 12 to 24wks, compared to a loss in strength in the EP+OO group.
[a,b] change in measure of strength from baseline is significantly different between groups with corresponding letters with, $p<0.05$

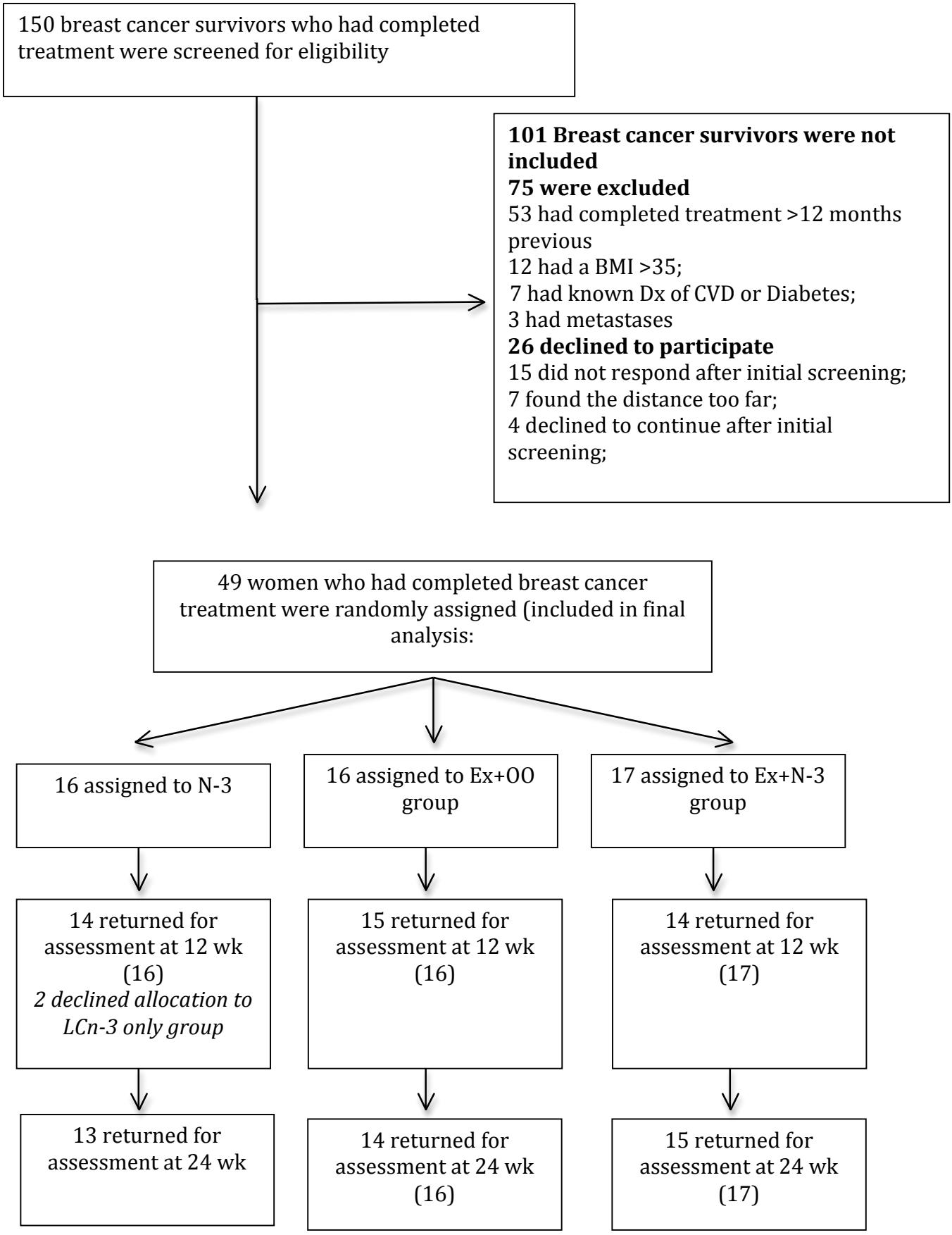


Figure 1. CONSORT diagram for participant flow through the intervention

N-3: Fish oil only; EP+OO: Placebo plus exercise and nutrition intervention; EP+N-3: Fish oil plus exercise and nutrition intervention. Numbers in ( ) indicate the number of participants included in the analysis

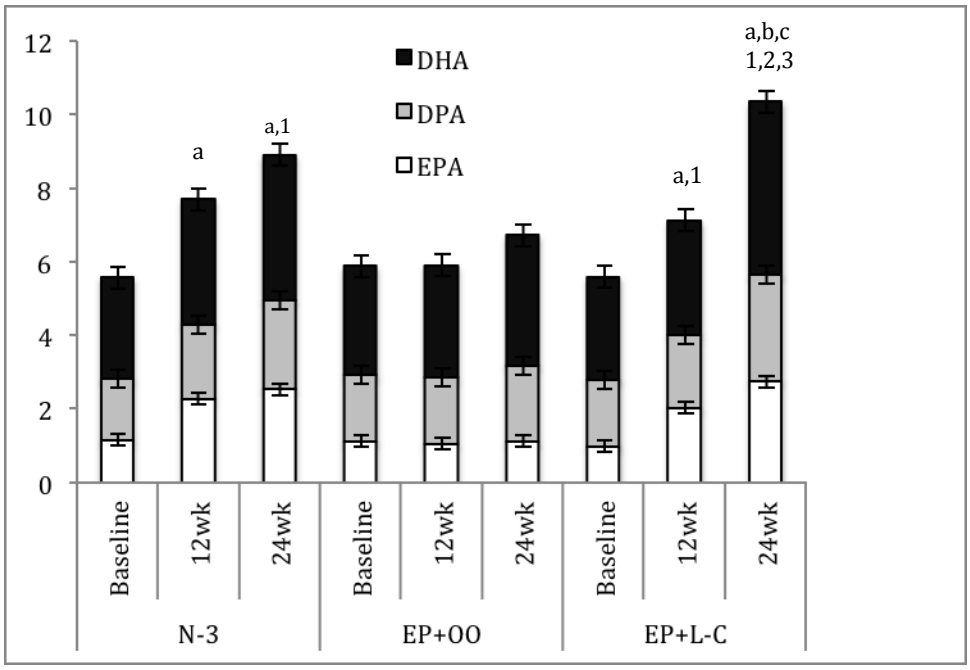


Figure 2. A significant oil x time interaction was observed ($p<0.05$), which indicated an increase in EPA for the fish oil supplemented groups. Significant within group changes from baseline identified numerically: 1=EPA, 2=DPA, 3=DHA, all $p<0.05$. Significant between-group changes from baseline in comparison to olive oil supplemented (EP+OO) group at the equivalent time point. a=EPA, b=DPA, c=DHA.

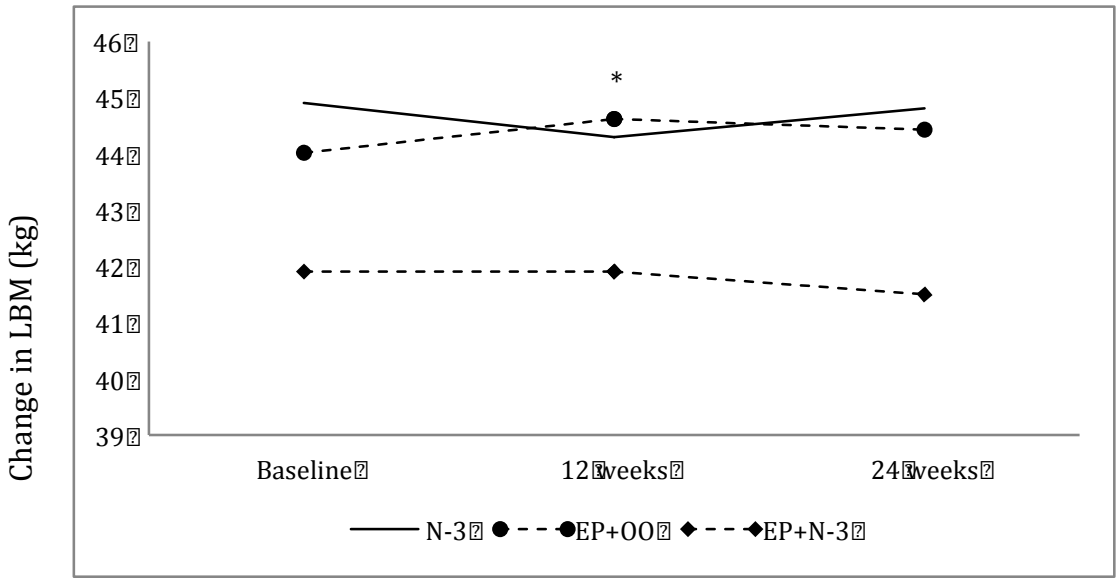


Figure 3. LBM for each treatment group at baseline, 12 and 24wks (n=49).
N-3: Fish oil only; EP+OO: Placebo plus exercise and nutrition intervention; EP+N-3: Fish oil plus exercise and nutrition intervention. LBM: Lean body mass. No significant interaction was seen for treatment x time; no significant difference was noted with groups for change in LBM. *EP+OO experienced a greater increase in LBM compared to LC, $p<0.05$

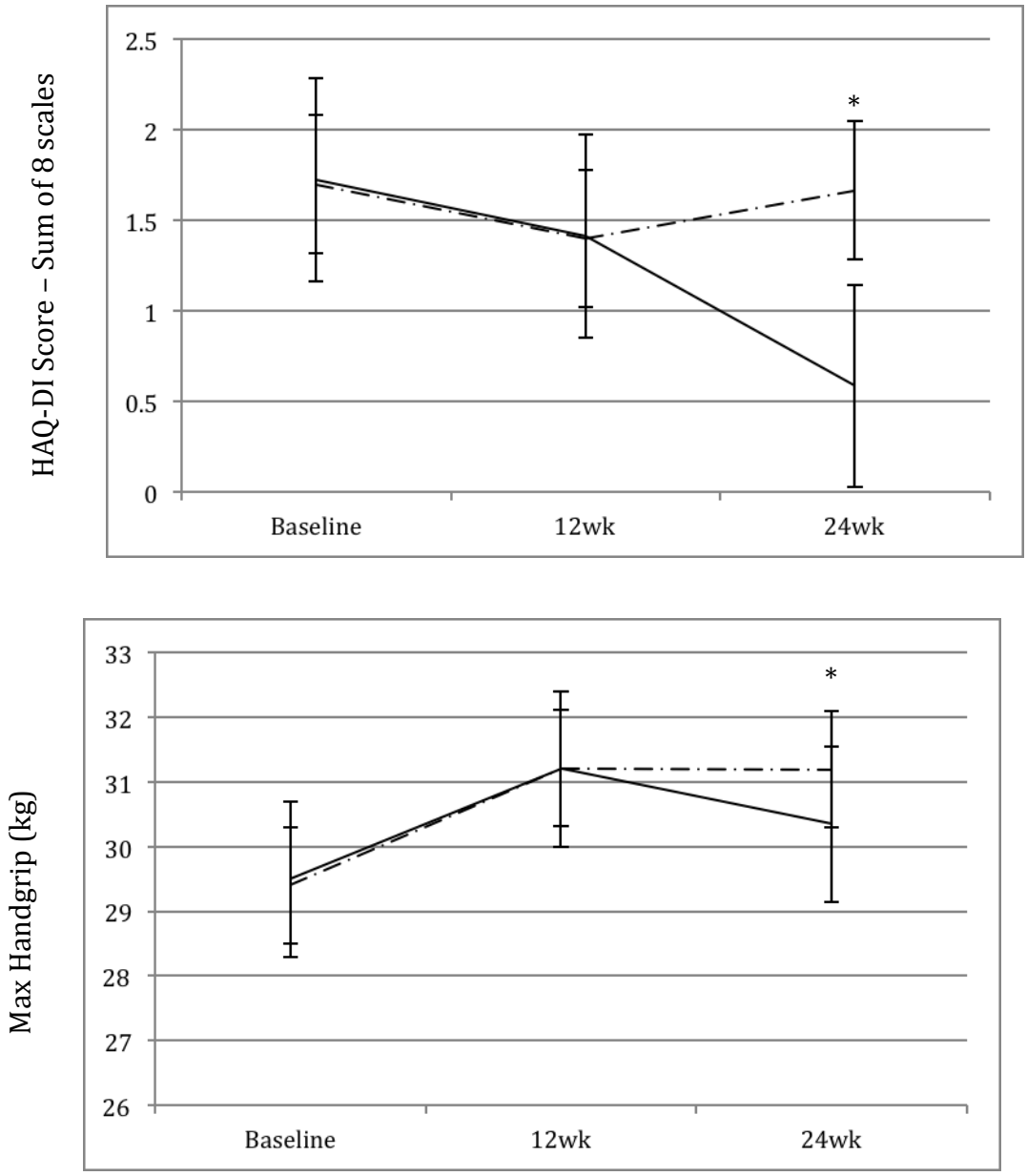
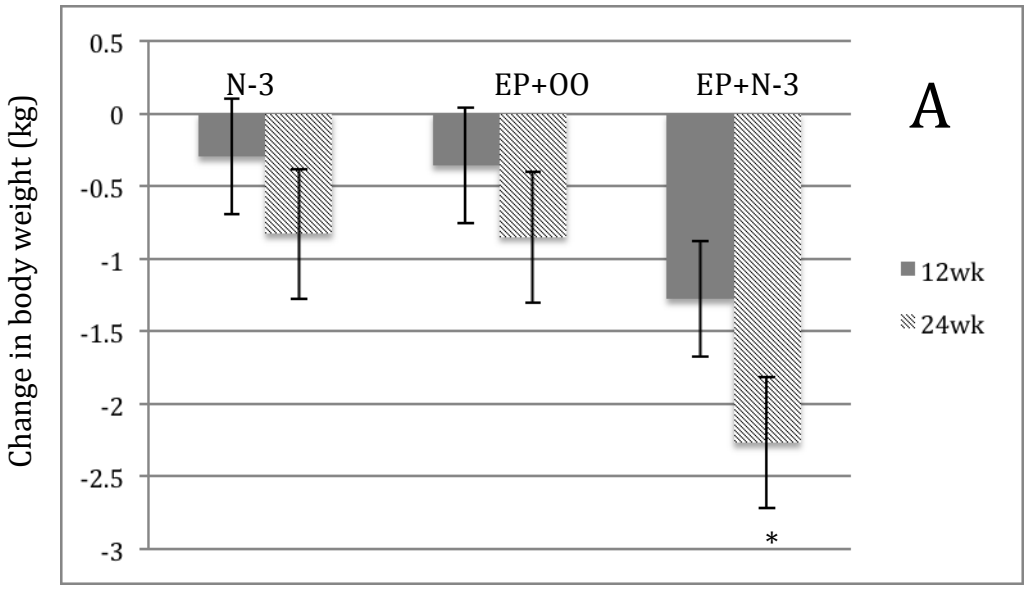
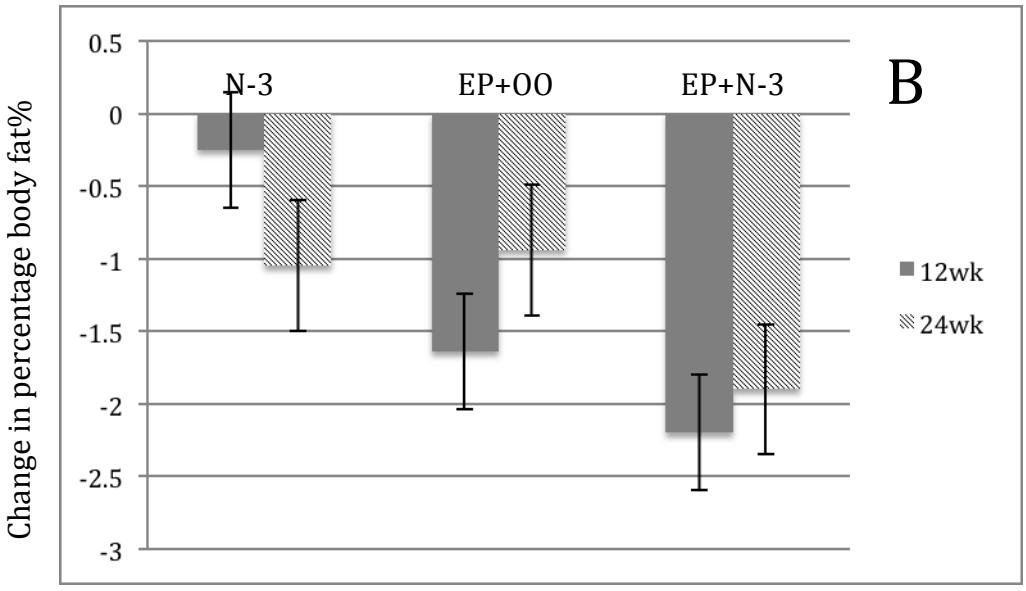


Figure 4. Mean (±SEM) of Health Assessment Questionnaire – Disease Index (HAQ-DI) (A) score and maximum handgrip strength of dominant hand (B) in those taking LCn-3(Dashed line ---; n=32) compared to those taking olive oil (Solid line –; n=16). N-3: Fish oil only; EP+OO: Placebo plus exercise and nutrition intervention; EP+N-3: Fish oil plus exercise and nutrition intervention. No overall interaction was observed for either measure over time. *A significant LCn-3 x time interaction was observed indicating a significantly different change between groups from 12 to 24 wk time points.


Change in body weight (kg)
0.5
0
-0.5
-1
-1.5
-2
-2.5
-3
N-3
EP+OO
EP+N-3
*
A
12wk
24wk


Change in percentage body fat%
0.5
0
-0.5
-1
-1.5
-2
-2.5
-3
N-3
EP+OO
EP+N-3
B
12wk
24wk

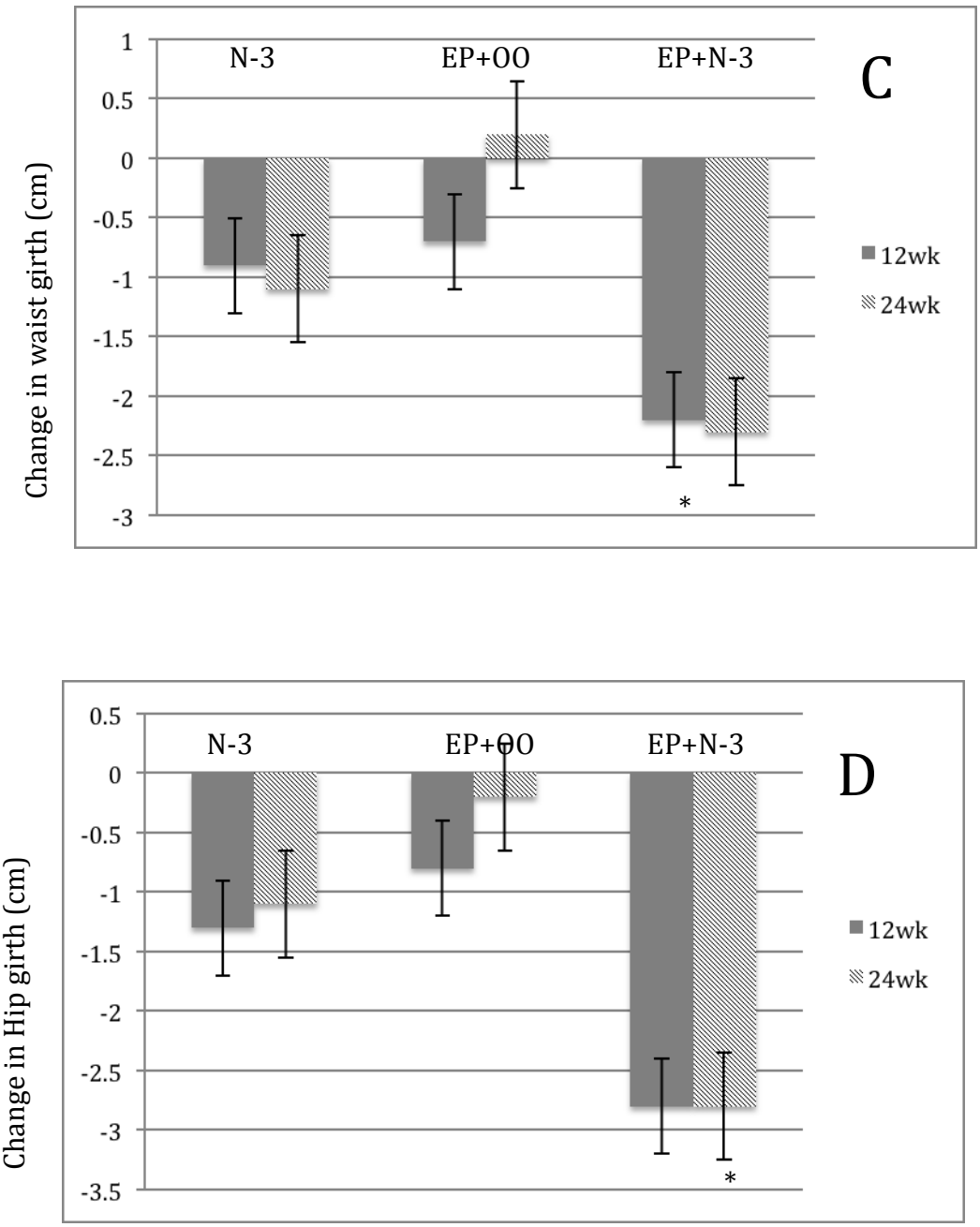


Figure 5. Change in body weight and measures of adiposity from baseline.
N-3: LCn-3 supplements alone; EP+OO: Olive oil and lifestyle program; EP+N-3: LCn-3 and program combined Graph **A**:. *: significant additive x time interaction ($p<0.05$), at 24 weeks, EP+N-3 experienced greater reductions in body weight compared to EP+OO and LC combined. **B**: A significant effect for time ($p=0.000$), no significant change in body fat% within or between groups. **C:** *significant additive x time interaction ($p>0.05$), EP+N-3 experienced greater reductions in waist girth at 12 weeks compared to EP+OO and LC combined; no significant difference at 24 weeks ($p=0.088$). **D:** *significant additive x time interaction ($p<0.05$), at 24 weeks, EP+N-3 experienced greater reductions in hip girth compared to EP+OO and LC combined.

5.2 Additional results and discussion

Results from measures that were not included in the manuscript but were included in the methods chapter are the dietary energy and protein intake, Greene Climacteric Scale, and Lymphoedema Index (L-Dex).

These measures were included as they are relevant outcomes to both LBM and body composition change, and quality of life after treatment for breast cancer.

5.2.1 Baseline associations

Change in energy and protein intake

No significant treatment x time, LCn-3 x time or exercise x time interactions were observed for energy intake (all $p>0.05$). There were no significant differences within or between groups for energy intake at any time point (Table 5.1). A non-significant treatment x time interaction was observed for protein intake, indicating a significantly greater increase in protein for the N-3 group compared to EP+OO and EP+N-3. However, when one outlier was removed from 24wk data (233g of protein/day), this relationship was no longer significant. Univariate ANOVA revealed that after excluding the outlier in the N-3 group, at 24 weeks, N-3 had a greater energy adjusted protein intake than EP+N-3 (96.9$\pm$5.5g vs. 78.2$\pm$4.9, $p=0.016$), with no differences present for EP+OO (89.0$\pm$5.1g, $p>0.1$ for both).

Relative protein intake and body composition change

Relative daily protein intakes were used to generate categories of protein intake (Figure 5.1). Cut-points of 0.8g and 1.0g/kg body weight (BW) were used, such that individuals who consumed above the threshold at baseline, 12wk and 24wks ('high') were compared to those who consumed less than the threshold on at least one occasion ('low'). For 0.8g/kg BW, there was no significant group x time interaction ($p=0.185$). However, pairwise comparisons noted that from 12 to 24wk time points, the low group tended to experience a greater reduction in LBM compared to the high group (-0.64kg vs. 0.19kg, $p=0.09$). No significant interactions were noted at any time-point, or for any pairwise comparisons.

TABLE 5.1. ENERGY AND PROTEIN (±SEM) INTAKE AT BASELINE, 12WK AND 24WKS IN N-3, EP+OO & EP+N-3 (N=49)

	Baseline		12wk		24wk		p-value*	p-value**
Energy Intake (kJ/day)	Mean	SEM	Mean	SEM	Mean	SEM		
N-3	6457	433	6486	609	6912	730	0.947	0.412
Ex+OO	6216	466	6396	524	6639	481	0.783	0.172
Ex+LCn-3	6837	380	6550	353	6523	344	0.246	0.414
Protein (g/day)								
N-3	84.1	4.7	79.8	4.0	94.9	5.7	0.946	0.566
Ex+OO	85.9	5.7	88.9	7.2	90.7	7.3	0.679	0.448
Ex+LCn-3	83.6	5.5	84	5	79.5	6.3	0.495	0.095

N-3: Fish oil only; EP+OO: Exercise and nutrition program plus olive oil; EP+N-3: Lifestyle program plus fish oil. No significant treatment x time interactions observed. *Within group change from baseline to 12wk time-point. **Within group change from baseline to 24wk time-point. Dietary intakes determined by a Dietitian led diet history questionnaire based on the previous months' food intake.

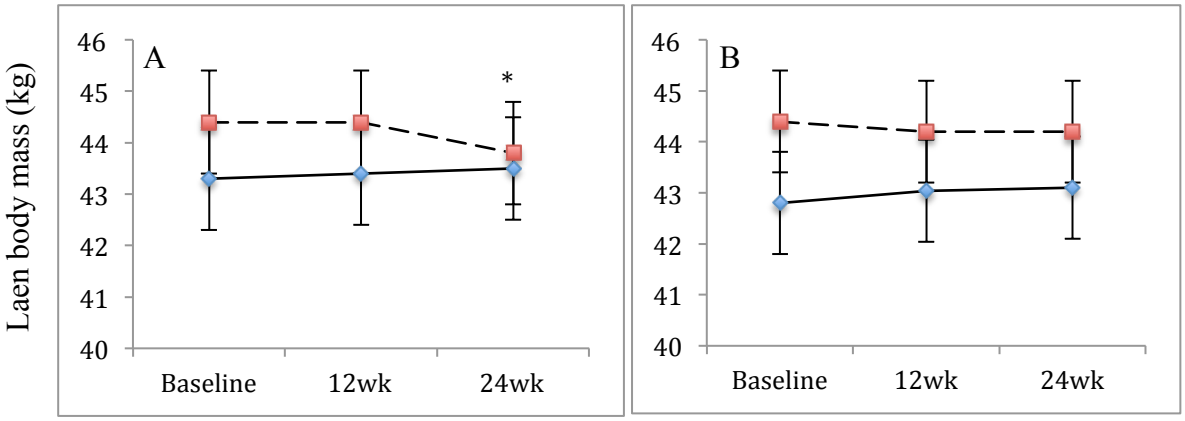


Figure 5.1 – Mean (±SEM) lean body mass at baseline, 12wk and 24wks (n=42).

A: Groups classified by daily consumption of protein of more or less than 0.8g/kg body weight; B: Classification by daily consumption of more or less than 1g/kg body weight. Dashed (---) represents group with at least one reported intake below classification threshold; Solid line represents group that reported intake above threshold at all time points. No significant interaction for group x time. *Non-significant trend for a greater decrease in LBM for those who consumed less than 0.8g/kg at least once compared to those who consumed more than 0.8g/kg at all time points.

Change in Greene Climacteric Scale (GCS) scores

No significant treatment x time interactions were observed for total GCS score, or any of the subscales (all $p>0.05$; Table 5.2). No change in association was observed when controlling for hormonal therapy ($p=0.398$) or menopausal status ($p=0.081$). However, with the exception of the Anxiety and Vasomotor subscales ($p=0.131$ and 0.09, respectively), a significant effect for time was observed for total GCS score and psychological, depression, somatic and vasomotor subscales (all $p<0.015$), such that all interventions improved symptoms of menopause. When defining groups as pre-, peri- or postmenopausal at baseline, or by hormonal therapy given (AIs, tamoxifen or no treatment), a significant effect for time was seen, with no differences in change between groups ($p>0.05$).

Change in L-Dex and incidence of lymphoedema

There were no significant treatment x time, LCn-3 x time or exercise x time interactions observed for L-Dex scores or incidence of lymphedema as determined by more than 10 (all p>0.05). In addition, there were no within group changes at 12 week or 24 week time points. Clinically significant L-Dex scores were indicated by a score of more than +10, or a change of +10 between assessments. At baseline, two individuals recorded a score of ≥10 (one each from EP+OO and EP+N-3 groups) with no differences in incidence between groups (chi-square: (p=0.602). Including those two, five participants experienced lymphedema at any point throughout the trial determined by an absolute score of ≥10, or a change of >10 from a previous time-point; one from EP+N-3, and two each from EP+OO and N-3 groups (chi-square: 0.459, p=0.795).

Compared to those who had an L-Dex score of more than +10 at baseline (n=2), there was no statistically significant differences for LBM for those with a score of less than 10 (44.8±1.3kg versus 43.5±5.7kg) or body fat% (43.9+7.9% versus 39.3+6.8%). Similarly, there were no significant differences in LBM or body fat% measurements when grouping participants into those who experienced an L-Dex score increase of 10 or more. Nor was there a significant correlation between L-Dex score and body fat% values at 12 or 24 weeks (p=0.923 and p=0.878, respectively).

5.2.2 Discussion of additional results

Overall energy and protein intake were consistent amongst groups at all three time points and both of these factors had no impact on body composition change over time. Previous studies have observed that energy intake is not associated with weight change after treatment for breast cancer (Demark-Wahnefried et al. 2001, Harvie et al. 2004). However, trials that have prescribed an energy deficit have consistently elicited body weight loss in breast cancer survivors (Thomson et al. 2010, Shaw, Mortimer, and Judd 2007a, Shaw, Mortimer, and Judd 2007b, Mefferd et al. 2007). Furthermore, a number of dietary interventions have shown that prescribing various models of healthy eating without energy restriction often creates smaller but clinically significant body weight loss (Chlebowski et al. 2006, Villarini et al. 2012, Hebert et al. 2001). However, Villarini et al noted that the healthy dietary changes resulted in an incidental decrease in energy intake that may explain weight lost (Villarini et al. 2012).

Epidemiological and clinical data indicate that LBM is influenced by protein intake, and that an adequate amount of protein may be required to maintain LBM over time (Bauer et al. 2013). In healthy populations, 0.8g/kg BW has been suggested as the recommended daily intake to maintain LBM status (Bauer et al. 2013). However recent evidence has indicated that this amount may be 1 to 1.3g/kg BW for older populations aiming for optimal LBM outcomes (Robinson et al. 2013, Bauer et al. 2013, Daly et al. 2012). At a lower threshold of relative protein intake (0.8g/kg body

weight), there was a trend for the low-intake group to experience a decline in LBM relative to those who consumed >0.8g/kg at all time points. However, no trends were noted when classifying groups by 1.0g/kg BW. To this point, no published data describes protein requirements for LBM maintenance in a breast cancer population and further research is needed to determine if there are significantly different requirements compared to a non-breast cancer population.
Overall, baseline and subsequent change in measures of menopausal symptoms, and risk of lymphedema were similar between groups.

All groups whether they were defined by treatment, menopausal states and hormonal therapy improved on Psychological, Somatic and Depression subscales of the GCS. No change over time was noted for Anxiety and Vasomotor scales, and no significant interactions were noted between groups. Biglia et al reported that in premenopausal breast cancer survivors, all subscales of GCS significantly worsened over 1-year of follow up (Biglia et al. 2010). Previous exercise and Yoga interventions have reported mixed findings in regards to perimenopausal symptoms. Two pilot Yoga studies (Waelde, Thompson, and Gallagher-Thompson 2004, Woolery et al. 2004) and one RCT (Chattha et al. 2008) have indicated improvement in perimenopausal symptoms and depression. However, Hayes et al (Hayes et al. 2013) reported no statistically or clinically significant change after an exercise intervention comparing face to face or telephone counseling.

Our population closely matched the norms of the GCS measured in a North Brisbane, Australia catchment (n=500) (Travers et al. 2005); such that the highest scores were noted in women aged 20 to 59 years, compared to the lower score for those 60 years and older. Thus, our population reported similar scores to that of perimenopausal women who have not been affected by breast cancer. Considering the normal decline in GCS related subscales, the significant effect for time for the overall population, and that all groups improved numerically in the majority of GCS subscales, indicates a positive effect for all interventions.

L-Dex scores were shown to be stable over the 24 week time period for all groups. The accumulative incidence of lymphoedema was 5 (10%), with only three individuals experiencing a score indicating clinically significant lymphoedema after baseline assessment. A large landmark safety study (Schmitz et al. 2009), which has since been confirmed by another (Cormie et al. 2013), reported that resistance training is safe for those with pre-existing lymphoedema and may reduce exacerbations compared to those who do not perform it, for both high and low load exercise (Cormie et al. 2013). Our results reflect that no increased risk was experienced for any group, yet

follow up may be too short to determine the true risk, as lymphoedema may occur years after treatment (Hayes et al 2009).

L-Dex measures had no significant impact on body fat% as measured by ADP. Body density can be affected such that a higher body fat% can be reported after consumption of more than 1000ml in the hour prior to measurement (Vukovich and Peeters 2003). For our study, compared to those with a 'normal' L-Dex score, i.e. <10), those who scored ≥10 at baseline had a significantly greater body fat%. However, the same magnitudes of difference were noted in hip and waist girths. Since, these measures are independent of upper arm swelling, it is likely that those with an L-Dex score of ≥10 had generally greater fat mass. Also, without data on absolute volume change, it could be speculated that the fluid accumulation in the arm did not significantly change body density due to it being less than 1000ml. These results are limited by sample size and should be confirmed in larger populations with individuals of varying severity of lymphoedema.

5.3 Summary of intervention findings

After 6 months, there was no change in LBM for any group, nor differences between them. Of interest though, only the group that were exposed to both the lifestyle program and the LCn-3 supplementation experienced significant decreases in body weight and markers of body fat. This is an important finding as body fat, particularly that found at the abdomen increases the risk of metabolic syndrome. Furthermore, the magnitude of body weight lost for the synergy group was similar that which was related to better survival in the WINS study (Chlebowski et al. 2006). LCn-3 was not found to influence LBM, cardiorespiratory fitness or strength-endurance. However, it was seen to better maintain physical function and strength as measured by the HAQ-DI and grip strength, respectively. These findings match up with a small number of studies that indicate physical functional improvements with LCn-3 intake (Hutchins-Wiese et al. 2013) and tissue levels of LCn-3 (Robinson et al. 2008).

Quality of life improved for all groups, however CRP was not significantly influenced by either LCn-3 intake or the lifestyle program. However, these findings did agree with previous research These findings are important for women who have been treated for breast cancer. While our sample was relatively small, this study provides the first evidence of specific nutrients being used in conjunction with exercise and nutrition advice to improve outcomes for breast cancer.

TABLE 5.2. INTERACTIONS FOR GREENE CLIMACTERIC SCALE, HAQ-DI AND L-DEX AT BASELINE, 12 AND 24WKS (N=49)

	Baseline		12wk		24wk			
GCS-Total	Score	SD	Score	SD	Score	SD	p-value*	p-value**
N-3	13.19	7.5	8.86	4.5	7.13	4.7		
Ex+OO	13.38	5.8	11.53	6.5	9.19	7.6	NS	0.000
Ex+LCn-3	14.88	11.4	12.07	8.3	10.00	8.6		
GCS-Psych								
N-3	5.44	4.4	6.07	4.6	2.75	2.7		
Ex+OO	6.13	3.3	8.07	5.6	3.38	3.4	0.942	0.000
Ex+LCn-3	7.47	5.8	9.73	7.2	5.00	4.7		
GCS-Anxiety								
N-3	2.81	2.3	2.00	1.0	1.69	2.0		
Ex+OO	3.25	2.0	2.67	2.0	1.88	2.0	0.841	0.131
Ex+LCn-3	4.10	2.8	3.50	3.0	3.20	2.7		
GCS-Depression								
N-3	2.63	2.5	1.86	1.7	1.06	1.1		
Ex+OO	2.88	1.7	2.53	2.2	1.50	1.8	0.919	0.004
Ex+LCn-3	3.41	3.3	3.33	2.5	1.82	2.4		
GCS-Somatic								
LCn-3	3.94	3.4	2.21	2.0	1.63	1.7		
Ex+OO	3.19	1.8	2.93	2.0	2.31	2.2	0.421	0.015
Ex+LCn-3	4.24	4.0	2.60	2.1	2.24	2.0		
GCS-Vasomotor								
LCn-3	2.38	2.2	1.93	2.1	1.75	2.1		
Ex+OO	2.50	1.9	2.27	2.0	2.56	2.5	0.708	0.09
Ex+LCn-3	2.24	1.9	1.40	1.5	1.94	2.0		
L-Dex								
LCn-3	0.34	3.96	-1.91	7.49	1.02	11.22		
Ex+OO	2.43	6.42	2.81	9.44	3.82	9.30	0.753	0.383
Ex+LCn-3	1.31	7.04	0.46	5.87	0.43	6.42		

N-3: Fish oil only; EP+OO: Exercise and nutrition program plus olive oil; EP+N-3: Lifestyle program plus fish oil; GCS: Greene Climacteric Scale; L-Dex: Lymphoedema index - <-10 or >10 is indicative of lymphoedema. P-value*: interaction for treatment x time; **: effect for time (overall change of population). No significant interactions found between groups.

Chapter 6 – Discussion, Future Direction and Conclusion

This chapter provides a full discussion and conclusion for the thesis as a whole. In order to interpret our results in the context of an Australian population, the generalisability of our sample to the larger population has been discussed. Furthermore, the limitations and strengths of our study are presented in order to validate the strength of the conclusions made and to aid in suggesting pathways for more research in this area.

6.1 Generalisability of the intervention study

Overall, our sample was representative of a young Caucasian Australian population. Women who were eventually included in the study were younger than those typically diagnosed with breast cancer in Australia (Australian Institute of Health and Welfare & Cancer Australia 2012, Statistics 2013). Compared to large representative US breast cancer interventions (Chlebowski et al. 2006, Pierce et al. 2002) and observational cohorts (Caan et al. 2012, Lynch et al. 2010), socioeconomic status (Australian Institute of Health and Welfare & Cancer Australia 2012), treatment, diagnostic, body composition and physical activity related characteristics of our sample were similar. Furthermore, the randomisation of our group was adequate with no significant differences at baseline amongst all primary, secondary and demographical variables.

6.1.1 Comparability of sample to general breast cancer population

The sample recruited into the MODEL study was younger (mean age: 48.9$\pm$ 1.39yrs) than the overall Australian average diagnosed with breast cancer in 2013 (Australian Institute of Health and Welfare & Cancer Australia 2012), and those included in an international prospective cohort study (n=12 915; mean age: 57$\pm$10.5yrs) (Caan et al. 2012). Australian breast cancer statistics indicated that 6%, 69% and 25% of breast cancers were diagnosed in those of ages <40yrs, 50-69yrs and >70yrs, respectively. Participants in the current intervention were distributed as 14.3%, 83.7% and 2%, respectively. Previous epidemiological research has shown that younger populations are more likely to perform higher levels of physical activity (Irwin et al. 2004), yet also have a higher risk of weight gain after treatment (Sheean, Hoskins, and Stolley 2012). The risk of greater weight gain noted in younger individuals may have been advantageous for the study. In a population that is more likely to experience adverse changes in body composition (Demark-Wahnefried et al. 2002, Demark-Wahnefried et al. 2001) a greater effect of a successful intervention may be noted.

The majority of our recruitment resources were directed through the Wesley Medical Centre, a private hospital in Brisbane. We observed that 63% of our participants self-reported an annual household income of >$80 000 and 59.2% had completed a University degree. According to the

Index of Relative Socioeconomic Disadvantage, these two factors are associated with more advantaged socioeconomic status (Statistics 2013). Australian data indicate breast cancer risk is increased in those in higher socioeconomic status, thus making our sample relevant to the local population (Australian Institute of Health and Welfare & Cancer Australia 2012). Our participants did have a higher University completion rate than those in the International pooling project made up of a North American population (Caan et al. 2012). From non-breast cancer populations, higher levels of education have been associated with higher levels of physical activity (Pan et al. 2009, Chad et al. 2005). However, despite the mixed findings with regard to education, objectively measured %time spent in sedentary and light intensity activity was closely matched with observational data of older breast cancer survivors (mean age: 69±13yrs) reported from the NHANES data (Lynch et al. 2010).

Of those included, 45% of the participants were employed full time, 87.8% were employed in some capacity and all were able to achieve 80% attendance. This suggests that the intervention may be transferable to working or non-working populations.
Our sample, of which 85% were diagnosed with Stage I and II breast cancer, had similar diagnostic characteristics to two large (n=2437; 94%(Chlebowski et al. 2006) & n=3088; 90% (Pierce et al. 2002)) intervention and cohorts and those included in the US category of the international pooling project (87.6%) (Caan et al. 2012). In addition, oestrogen receptor status, treatment rates of chemotherapy and radiation therapy were comparable to results reported by Chlebowski et al (2006) (Chlebowski et al. 2006), however, slightly higher rates of radiation therapy than those reported by Pierce et al (2002)(Pierce et al. 2002) (67% vs 48.4%, respectively).

6.1.2 Adequacy of randomisation

Across the three intervention groups, there were no differences between any of the primary or demographical variables. Even though the sample size was smaller than planned, the randomisation was sufficient to appropriately allocate participants evenly.

Overall, while our sample was younger than those typically diagnosed with breast cancer in Australia, these differences did not have seem to have an effect on physical activity, treatment and diagnostic variables compared to previous intervention and observational trials. In addition, the randomisation appropriately allocated an even distribution of demographical attributes. Taken together, results from our population are generalisable to a predominantly Caucasian population of women diagnosed with breast cancer

6.2 Limitations & Strengths of the study

6.2.1 Limitations

It is acknowledged that our study may have been limited by not including a placebo-only control group. While this may have allowed a comparison of the effect of LCn-3 alone versus no intervention, the Uniting Care Health HREC did not approve the study design with this group. In addition, this study was designed to test the efficacy of LCn-3 compared to a lifestyle intervention or a combination of change in LBM. It has been previously established that exercise maintains and, in some cases, increases LBM. Thus to answer our hypothesis, the study only required the comparison of best practice nutrition and exercise prescription from current guidelines, and best practice prescription in combination with LCn-3, and did not require a placebo only group. The LCn-3 only group was added to determine if tailored exercise prescription is needed to influence body composition change, or if this occurs due to the increased awareness physical activity benefits after diagnosis.

Due to slower than expected recruitment, we were unable to reach projected participant numbers. The lower than expected recruitment rate was likely due to there being a number of other trials concurrently recruiting participants with similar demographic attributes, and that our eligibility criteria were too narrow. In addition, our main recruiting consultants from the Wesley Medical Centre unfortunately experienced a significant decrease in eligible patients at the time of recruitment. A number of other recruitment strategies were added, such as via advertising through radio, social media, additional hospitals, and this may have introduced a more representative sample than if all participants were recruited from the one private medical facility. On the other hand, the clinically and statistically significant effects of LCn-3 on body composition (Munro and Garg 2012) and muscle function (Smith et al. 2011b, Rodacki et al. 2012) have been reported within similarly sized samples. The lack of effect for LBM seen between groups in our study may be a result of the lower power, however, our results are similar to those with studies that included a larger population with equivalent intervention protocols (Demark-Wahnefried et al. 2008, Mefferd et al. 2007, Djuric 2011, Matthews et al. 2007, Schmitz et al. 2009, DeNysschen et al. 2011). Alternatively, it is possible that the results reflect the true effect of the intervention, which supports LBM maintenance after all interventions, and an improvement in adiposity for participants exposed to both LCn-3 and the lifestyle program.

To improve recruitment for future studies it is suggested that researchers form strong associations with a large number of oncologists and oncology teams. The most direct and effective method of recruitment was through local clinicians, and developing a strong network of these professionals

would make recruitment more consistent and timely. Social media, research networks and breast cancer groups were less effective, albeit these avenues should be explored to maximise the recruitment potential.

Prescription of home-based resistance training using body weight and elastic resistance equipment may have been inadequate to elicit an appropriate anabolic response. The one study that has reported significant functional improvements due to LCn-3 and exercise used specifically prescribed lower leg resistance training using a gym facility (Rodacki et al. 2012). It is possible that even though our participants were asked to perform the exercise until temporary fatigue, this training prescription may not have been performed adequately when participants were unsupervised. Since Rodacki et al (2012) (Rodacki et al. 2012) did not measure LBM change, it is possible that LCn-3 is only effective in enhancing LBM power in conjunction with resistance training, and may not have significant effects on LBM accretion. A previous study in healthy young males indicated that exercising to temporary fatigue elicited the same response in MPS regardless of the load (Burd et al. 2010). Again, this study was conducted under supervision on weight machines, but only over one bout of exercise. Prolonged exercise prescription encouraging this type of exercise may not be sustainable, or fully adhered to over longer periods of time.

6.2.2 Strengths

Our study is strengthened by the double blind randomised control design, high quality objective and validated measures, intention to treat analyses and long term follow up to determine adherence to lifestyle changes after active support has ceased.

Intervention and study design

The double blind randomised controlled trial design is considered the 'Gold Standard' for assessing the difference in efficacy of two or more treatment protocols. In addition, our study design was pragmatic for two reasons: it could be replicated in general practice at low cost; and, the participants in the third intervention arm that prescribed LCn-3 supplementation alone allowed participants to exercise and diet freely, as opposed to restricting their behaviour. These two points allow us to report on the feasibility and efficacy of interventions, and to determine which protocol will be most beneficial to practitioners in aiding breast cancer survivors in body composition change. We found that those who did not receive specific exercise prescription finished the intervention performing similar amounts of aerobic physical activity as those who were prescribed resistance and aerobic exercise. This suggests that general awareness of physical activity after breast cancer may be sufficient to motivate individuals to increase aerobic exercise without specific

advice. Or, the nature of the trial attracted individuals who were already willing and wanting to exercise.

The diet (Robien, Demark-Wahnefried, and Rock 2011) and exercise (Schmitz et al. 2010, Hayes et al. 2009) intervention were based on best evidence for breast cancer survivors, and the content was delivered by a dual qualified Accredited Exercise Physiologist and Accredited Practising Dietitian (Primary Investigator – CM).

The dose of LCn-3 supplementation prescribed was one that has previously produced the highest uptake into erythrocytes over a 2 to 6 month period (Yee et al. 2010) regardless of BMI. BMI has been negatively associated with erythrocyte concentration of LCn-3 after supplementation in children (Hogg et al. 2006). Furthermore, the LCn-3 supplements used can be found commercially adding to the pragmatic design.

Follow up to six months after baseline allowed the long-term adherence and subsequent changes in body composition to be measured. Detraining in previous studies has led to a decrease in strength, fitness and quality of life (Herrero et al. 2007). Our results show that strength was maintained through 6 months, and there was an overall trend for improved body composition in all groups .

Outcome measures

Our primary outcome of body composition was analysed using the BOPOD (CosMed, USA), which has been previously validated against under-water weighing (Lukaski 2009). To compliment this measure, waist and hip girths were also measured and responded comparably to changes in weight and body composition measured by the BODPOD. In addition, the presence of lymphedema did not seem to alter body composition results, however validation studies in breast cancer populations with varying severity of lymphoedema need to be conducted to confirm these results.

Measurement of erythrocyte LCn-3 content is a reliable marker of long term (2 to 6 month) LCn-3 supplementation and is able to determine differences in dosage (Yee et al. 2010). In addition, blood analysis is removed from estimation errors inherent in intake calculations derived from diet history questionnaires (Martin 2004).

Similarly, serum hs-CRP is an established marker for chronic inflammation, which we hypothesised as a potential mechanism for LBM wasting.

Major potential confounders of body composition are dietary intake and physical activity. The interviewer administered Diet History Questionnaire has been previously validated and incorporates a one-on-one investigation with an Accredited Practising Dietitian (Martin 2004). This method of determining dietary intake has been shown to be less disruptive to normal eating patterns than weighed food records, and when assessing food intake of more than 7-days, is appropriate for assessing individual and group changes in eating patterns (Rutishauser 2005).

Physical activity was measured in three different ways: objective monitoring with uniaxial accelerometry, and subjective reporting via the Active Australia Questionnaire and physical activity diary provided as part of the intervention. The findings from the literature review indicate that women who have been treated for breast cancer experience significant adverse changes in their body composition. Our observational study indicates that muscle function, as measured by upper body strength-endurance and cardiorespiratory fitness, are strongly associated with measures of body composition after treatment. The randomised controlled trial indicated that consumption of LCn-3, a 12-week exercise and nutrition lifestyle program, and LCn-3 plus the lifestyle program all promote LBM maintenance, have minimal effects on CRP, and promote better quality of life over time. The combination of LCn-3 plus the lifestyle program may be more effective in reducing body weight and markers of adiposity compared to either intervention alone. In regards to muscle function, structured semi-supervised training improves upper body muscular strength-endurance, while LCn-3 consumption may promote better maintenance of grip strength and physical function independent of structured exercise. These results warrant further research using a larger population over a time period relevant to post-treatment cardio-metabolic outcomes and physical function outcomes.

6.3 Body composition change after breast cancer

6.3.1 Body composition changes after treatment: patterns and mechanisms

The published and unpublished sections of Chapter 2 composing the literature review of this thesis were used to create our theoretical model. The current evidence indicates that body weight gain in conjunction with a reduction in LBM is common after treatment for breast cancer (McDonald, Bauer, and Capra 2011). Furthermore, body weight and adiposity increases are more likely to occur after chemotherapy, as a result of lower physical activity, in those of younger age and/or in those who are premenopausal at diagnosis (Sheean, Hoskins, and Stolley 2012). In breast cancer survivors, loss of LBM is potentially related to chemotherapy treatment (Mourtzakis and Bedbrook 2009), yet there is far less direct evidence of this compared to changes in body weight and body fat%. On the other hand, Murphy et al observed a significant LBM wasting effect of chemotherapy in non-small cell lunch cancer (NSCLC) patients (Murphy et al. 2011). They reported that prevalence of myopenia at baseline and chemotherapy and cancer related LBM wasting were correlated with the concentration of plasma EPA. In contrast, strong prospective evidence has linked Aromatase Inhibitor use with an increase in LBM over time (van Londen et al. 2011, Montagnani, Nuti, et al. 2008).

The results from our cross sectional analysis indicates that increasing levels of moderate intensity physical activity, greater upper body strength and greater cardiorespiratory fitness are strong

independent predictors of weight and age adjusted LBM. This is the first study to report that markers of LBM function are attributed to LBM status after treatment, and that a threshold effect may exist for each measure in relation to adjusted LBM. No data in breast cancer populations is currently available to compare these findings, and without follow up it is difficult to know if these thresholds are clinically relevant to obesity-breast cancer related disease risk. However, a substantial literature base indicates that improved longevity and quality of life are related to greater cardiorespiratory fitness (Gau et al. 2010, Lee, Blair, and Jackson 1999), and LBM function (Newman et al. 2006, Ruiz et al. 2008), respectively. Thus, this is a finding worth further investigation.

We did not see a significant effect of LCn-3 on measures of body composition at baseline. Our data indicated non-significant, yet opposite effects of EPA and DHA on measures of physical function, which cannot be fully explained by further analyses and may be Type II error. It is possible these results are spurious as the results pertaining to EPA are in direct contrast to a large body of evidence that report a benefit for this nutrient.

Clinically, decreases in LBM and increases in adiposity, particularly visceral adiposity (Cheney, Mahloch, and Freeny 1994) with or without total body weight increase, is a negative outcome for women after breast cancer treatment. Higher body fat% is associated with increased circulating inflammatory molecules (Dee 2010), which is predictive of greater non-breast cancer and overall mortality (Pierce, Ballard-Barbash, et al. 2009). Furthermore, due to improvements in treatment, cardiovascular mortality is more common than breast-cancer related deaths in survivor populations (Hanrahan et al. 2007). Hence, the close relationship of body composition change to cardio-metabolic risk in this population exposes the importance of addressing healthy body weight change in this population.

In terms of non-modifiable risk factors, we did not find significant effects on LBM for differing types of treatment (chemotherapy/radiotherapy or aromatase inhibitors), or menopausal status. Thus, with regards to our theoretical model, our results are in contrast to hypothesized (Mourtzakis and Bedbrook 2009).and previously observed findings (Demark-Wahnefried, Campbell, and Hayes 2012, Goodwin et al. 1999) that have suggested a modifying effect of menopausal state and treatment. A difference and limitation of our study was that our cross-sectional analyses would not be sensitive to longitudinal change where these relationships were previously identified (Harvie 2010, Montagnani, Gonnelli, et al. 2008, Francini et al. 2006). On the other hand, our observed relationships between markers of physical activity and body composition agree with previous research. Irwin et al (Irwin et al. 2005) reported an inverse association between time spent performing sports/recreational activity and body fat%, however the authors made no comment of

longitudinal change in LBM. In practice, these results will strengthen the case for exercise training in the post-treatment period.

After a diagnosis of breast cancer, current guidelines recommend regular physical activity to optimise outcomes in relation to survival, ameliorating treatment related side effects, quality of life and risk of co-morbidity (Schmitz et al. 2010). Early in the post-treatment period is thought to be the most teachable time for promoting behaviour change in relation to dietary and exercise habits associated with reduced risk of ongoing disease risk (Rabin 2009). Our results further confirm that physical activity was shown to enhance cardiovascular fitness, and with upper body strength was important to a greater LBM and lower fat mass. LBM is an important consideration for ongoing health in the general population (Pedersen and Febbraio 2012). Furthermore, LBM function, i.e. muscle strength and cardiovascular fitness, may be a more important indicator of survival (Newman et al. 2006, Ruiz et al. 2008). LBM function is more closely related to falls risk, the ability to perform activities of daily living and participation in exercise than LBM alone. This paper aids in connecting LBM function and body composition in a breast cancer population.

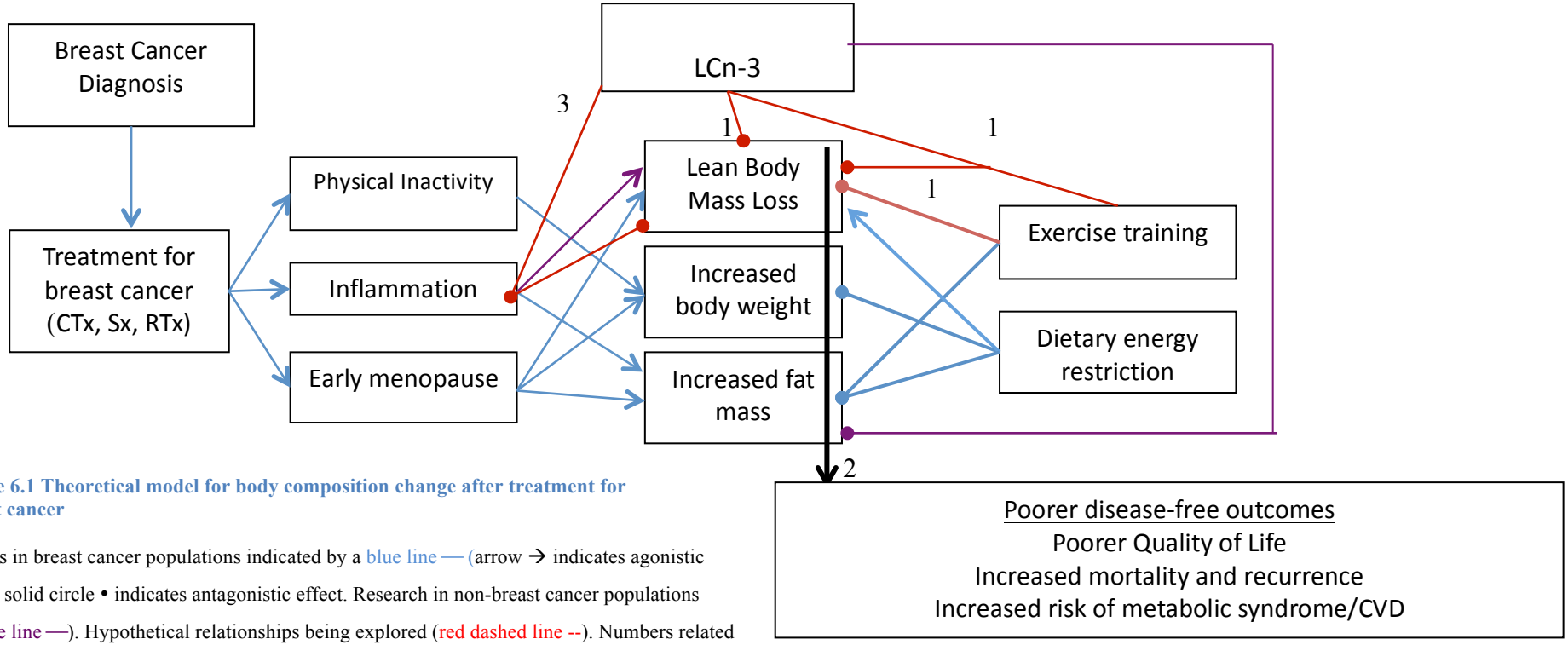


Figure 6.1 Theoretical model for body composition change after treatment for breast cancer

Studies in breast cancer populations indicated by a blue line — (arrow → indicates agonistic effect; solid circle • indicates antagonistic effect. Research in non-breast cancer populations (purple line —). Hypothetical relationships being explored (red dashed line --). Numbers related to hypotheses: 1: The combination of LCn-3 and exercise training promotes greater LBM accretion than either intervention alone; 2: LCn-3 will reduce inflammation (C-reactive protein), which will be related to a reduction in loss of LBM; 3: Improved body composition as a result of LCn-3 and exercise training will improve quality of life.

6.4 Primary and Secondary Hypotheses

6.4.1 Primary Hypothesis

Our primary hypothesis (1 - Figure 6.1)- was that the individuals randomised to the group, which combined LCn-3 and nutrition and exercise program would experience significantly greater increases in LBM than those exposed to LCn-3 or the program alone. This hypothesis was not accepted. Our results indicate that LBM change was equivocal for all groups, with all intervention groups experiencing LBM maintenance.

LBM change in breast cancer related trials

Our results agree with the majority of exercise and exercise plus nutrition interventions that show no increase in LBM over time (Matthews et al. 2007, DeNysschen et al. 2011, Battaglini et al. 2007, MacVicar, Winningham, and Nickel 1989, Burnham and Wilcox 2002, Guinan et al. 2013, Schmitz et al. 2009, Mefferd et al. 2007, Djuric 2011). However, those that have reported LBM growth have typically been conducted in a gym, and exercise programming was supervised at least twice per week (Irwin, Alvarez-Reeves, et al. 2009, Schmitz, Ahmed, et al. 2005, Herrero et al. 2006, Fernández-Lao et al. 2013). Prescription of semi-supervised moderate intensity aerobic activity with elastic band resistance exercises may not have been adequate to elicit an appropriate anabolic response in our population. These findings mirror those of previous trials using the same type of resistance training equipment (Demark-Wahnefried et al. 2008). However, given the predisposition of breast cancer survivors to experience loss of LBM due to treatment and activity related factors (Sheean, Hoskins, and Stolley 2012, Mourtzakis and Bedbrook 2009), any level of aerobic or resistance training intensity that enables maintenance of LBM in conjunction with total weight loss or stability may be considered a good outcome.

In regards to LCn-3, our results agree with those of trials in non-cancer populations that have shown no effect on LBM change after LCn-3 supplementation with or without dietary energy restriction (Couet et al. 1997, Noreen et al. 2010, Crochemore et al. 2012, Munro and Garg 2012, Storlien et al. 2001, Hlavaty et al. 2008, Krebs et al. 2006, Abete et al. 2008), or in conjunction with aerobic exercise training (Hill et al. 2007). In contrast, for individuals experiencing cancer related loss of LBM, higher plasma LCn-3 levels and LCn-3 supplementation during treatment, has resulted in the attenuation of LBM loss, while non-supplemented control groups have continued to rapidly lose body weight and LBM (Murphy et al. 2011, Fearon et al. 2003, Fearon et al. 2006). Given these contrasting findings between populations, the effect of LCn-3 may only be clinically evident in populations experiencing extreme LBM and significant reductions in tissue LCn-3 content. Breast cancer survivors seem to have a metabolic health profile that is more similar to those with cardio-

metabolic chronic disease populations (Healy et al. 2010, Thomson et al. 2005, Pierce, Neuhouser, et al. 2009), with clinically significant yet slower loss of LBM (<2kg per year(Harvie et al. 2004)) than those with advanced cancer.

Similar to Denysschen et al (2011), the group randomised to capsule only (N-3) performed a similar amount of exercise as those who participated in the lifestyle intervention. The increase in physical activity may have prevented loss of LBM in this group thus decreased our ability to observe between group differences. Physical activity after treatment for breast cancer is becoming more widely supported by both the medical community and media, with specific exercise and breast cancer awareness initiatives being run in the community (National Breast Cancer Foundation 2012). This may place positive pressure on women who have completed treatment to continue or commence physical activity. Secondly, it is possible that participants volunteering for an exercise and nutrition this trial may have already been more inclined than the general population to engage in healthy eating and physical activity. Or finally, knowledge of their participation in an exercise and nutrition trial measuring physical function and body composition markers may have provided additional motivation for them to improve their current habits. In essence, our findings suggest that due to baseline physical activity levels of a younger Australian breast cancer cohort, tailored exercise prescription resulted in equivocal volume of exercise as control group who were given no restrictions on activity levels. Furthermore, exercise was similar across groups at the end of follow up, yet significant differences were found for erythrocyte LCn-3. Thus, in our study LBM change is likely to be most related to exercise training rather than an interaction of the LCn-3 and exercise training.

6.4.2 Secondary hypothesis – Quality of life

Our secondary hypotheses, that the combined intervention group would experience greater improvements in quality of life and inflammation (2- Figure 6.1), were not accepted. Compared to baseline, after 12 and 24 weeks, QOL improved in all groups with no difference between them. Furthermore, no association was noted between erythrocyte LCn-3 content and change in QOL score.

Quality of life after breast cancer trials

Overall quality of life (Ohira et al. 2006, Herrero et al. 2006) and physical function (Courneya et al. 2007), breast (Fernández-Lao et al. 2013), and psychosocial (Ohira et al. 2006, Matthews et al. 2007) subscales have been shown to improve after exercise-only interventions. In opposition to this, previous exercise and diet combined studies of similar methodology to ours have not shown significant between group improvements in QOL (Demark-Wahnefried et al. 2008, Djuric 2011). Herrero et al (2007) reported that QOL improvements seen after an eight-week combined aerobic

and resistance training intervention returned to baseline after a subsequent 8 weeks of detraining. Additionally, suggestions from Hebert et al (2001) who reported findings from a diet-only intervention in breast cancer survivors may be applicable to our results. They suggested that initial improvements in lifestyle habits during the 12-week nutrition and exercise program may have contributed to those participants setting a higher standard of behaviour for themselves. After regular meetings were ceased during the 12 to 24 week interval, and a partial return to their original pre-intervention habits occurred, it may have caused personal disappointment, and thus a decrease in subjective QOL. This phenomenon would not have impacted the LCn-3 alone group as no expectations were placed on their activity levels at any point. Furthermore, Hsu et al (2013) examined a prospective cohort for QOL of life changes. They reported that 1 year after breast cancer diagnosis, women in general experienced 5.6% improvements in their QOL without intervention (Hsu et al. 2013). Previously, a meta-analysis revealed that cardiorespiratory fitness was positively associated with QOL as measured by FACT-B (McNeely et al. 2006). Participants in the LCn-3 only group did not experience a significant increase in cardiorespiratory fitness, however they did show improvements in upper and lower body strength, which corroborated with increases in reported participation in physical activity. Therefore, their improvement in physical function in the absence of expectations and standards that were placed on the tailored exercise and nutrition groups, may have contributed to the LCn-3 only group realising a continued increase in QOL over 24 weeks. Unstructured anecdotal feedback from the participants of the nutrition and exercise program matched these results in that the intervention was well received; the lack of accountability in weeks 12 to 24 reduced their motivation to perform the full list of exercises.

In summary, overall and physical function measures of QOL may improve after exercise training compared to non-exercising controls, however increasing time from diagnosis also seems to increase markers of QOL. LCn-3 did not enhance QOL overall, however continued follow up through phone or group support is an important consideration for future trials aiming to maintain and continue improvements in QOL.

6.4.3 Secondary hypothesis – Chronic Inflammation

We hypothesised that LCn-3 supplementation in conjunction with a tailored exercise and nutrition program would promote greater reductions in CRP compared to those who received LCn-3 supplementation or the program alone (3 – Figure 6.1). This hypothesis was no accepted. All intervention groups experienced no change in CRP after 24 weeks. While the lifestyle program plus olive oil group experienced a greater reduction in CRP than lifestyle plus LCn-3, this was not maintained long term. Our hypothesis was based on the premise that chronic inflammation contributes to LBM wasting in breast cancer populations (Mourtzakis and Bedbrook 2009). We

postulated that a combination of exercise and LCn-3 might reduce LBM wasting better than either in isolation, due to both having previously been shown to be anti-inflammatory.

6.4.3.1 Exercise and LCn-3 and change in CRP

In general, studies examining the effect of aerobic exercise on CRP in breast and non-breast cancer populations have typically reported significant benefit within exercise groups (Fairey et al. 2005, Guinan et al. 2013), but not between exercisers and controls. One trial in breast cancer survivors indicated a non-significant reduction in CRP after diet-induced weight loss (Fairey et al. 2005). The majority of research examining CRP change and LCn-3 indicates a strong cross sectional association with little to no effect for LCn-3 on CRP after supplementation. However, there is consistent suggestion in all three areas that significant benefit of intervention exists for those with higher levels of CRP at baseline.

Exercise and CRP

Smith et al (1999) conducted an uncontrolled six month aerobic exercise trial for those at risk of CVD and reported CRP was reduced by 35% overall, with 50% reductions for those in the highest quartile of CRP at baseline (Smith et al. 1999). Findings for change in CRP in breast cancer populations have shown similar effects for aerobic exercise on CRP. Two aerobic exercise trials of 15 (Fairey et al. 2005) and 12(Guinan et al. 2013) weeks, respectively, in breast cancer populations reported within group decreases in CRP. However, only a trend between exercise and control groups was reported for both studies (Fairey et al. 2005, Guinan et al. 2013). Fairey et al (2005) and Guinan et al (2013) reported a change in CRP of -1.39mg/L and -0.65mg/L, for exercise groups respectively, and stability for control, +0.01mg/L and -0.01mg/L, respectively.

Diet and CRP

In a dietary study, body weight loss of 6kg from energy restriction alone accompanied a non-significant decrease in CRP for breast cancer survivors (Thomson et al. 2010). In contrast, weight loss in non-breast cancer populations has been associated with significant decreases in CRP (Belalcazar et al. 2013, Yatsuya et al. 2011, Haffner et al. 2005, Tamakoshi et al. 2003, Kopp et al. 2003, Esposito et al. 2003). However, these studies have been conducted in individuals with higher baseline BMI, and have reported greater weight loss (>10% of baseline body weight) resulting from the intervention than the 7.5% reduction in body weight reported by Thomson et al (2010). In a population of breast cancer survivors, compared to those with a body fat% of less than 35%, those with a body fat% higher than 35% had significantly greater CRP values (Dee 2010). Considering increased adiposity is related to greater CRP through an increased release of inflammatory adipokines (Lee et al. 2009), significant effects therefore, may be more readily elicited for those with excessive adiposity. This has been confirmed when comparing greater CRP reductions for

overweight/obese populations and no changed for healthy weight populations after dietary manipulation (Neuhouser et al. 2012).

Finally, in confirmation of previous findings, after a 12-month diet and exercise intervention in breast cancer survivors, CRP was only reduced in the group with the higher baseline CRP (3.6mg/L vs 1.8mg/L). However, a caveat in this finding is that differences between groups was not analysed due to inadequate power.

LCn-3 and CRP

Despite strong cross sectional association of CRP and LCn-3 intake, trials prescribing LCn-3 supplementation have mixed effects on change in CRP over time (Smith et al. 2011a, Muhammad et al. 2011, Chan et al. 2002, Munro and Garg 2012, Madsen et al. 2003, Tsitouras et al. 2008, Micallef and Garg 2009).

Studies have shown that a CRP has an inverse relationship with markers of medium (Poudel-Tandukar et al. 2009) and LCn-3 intake (Kalogeropoulos et al. 2010, Micallef, Munro, and Garg 2009, Farzaneh-Far et al. 2009, Niu et al. 2006). However, five intervention studies (Smith et al. 2011a, Muhammad et al. 2011, Chan et al. 2002, Munro and Garg 2012) including a dose response investigation (Madsen et al. 2003) have reported no effect for LCn-3 on CRP levels. Similar to exercise and diet intervention literature, Tsitouras et al (2008) noted that a reduction in CRP was only found in those with higher baseline CRP levels (>3mg/L) (Tsitouras et al. 2008). Furthermore, Micallef et al (Micallef and Garg 2009) noted 39% ($p=0.009$) reductions in CRP after a blinded cross-over trial in men and women with hyperlipidaemia, relatively low BMI (mean: 26.6kg/m^2), and moderate CRP levels (2.9 to 3.2mg/L).

This is the first study to investigate the effects of LCn-3 on CRP in a breast cancer population to date. CRP has been correlated to increased incidence of cardiovascular disease and decreased survival in breast cancer survivors (Pierce, Ballard-Barbash, et al. 2009). Aerobic exercise (Fairey et al. 2005, Guinan et al. 2013), diet-induced weight loss (Thomson et al. 2010), and a healthy dietary advice plus exercise intervention (Djuric 2011) have resulted in statistically non-significant trends for a reduction in CRP. Similar to non-breast cancer populations, in our population, LCn-3 supplementation did not affect group based CRP values over the course of the intervention. In contrast, all groups significantly increased physical activity, subsequent markers of upper and lower body strength, and as a whole experienced a statistically significant decrease in CRP from baseline to 24 weeks. It is important to note that our sample was of lower BMI and had lower CRP levels at baseline than those studies that reported benefit for those with higher BMI and CRP levels.

Summary of CRP

Our population had a relatively low baseline CRP (mean: 1.75mg/L), BMI (mean: 26.3kg/m^2) and body fat% (29.7%). Therefore, considering previous data in weight loss and CRP, noting no significant change in CRP was not unexpected.

6.4.4 Change in body weight, fat%, waist and hip girths

The combination of LCn-3 and the lifestyle program was more effective in reducing body weight, waist and hip girths than either intervention alone. Change in body fat% was not significant between or within groups. In breast cancer survivors, high body fat% (Protani, Coory, and Martin 2010) and increased waist girth (Protani, Coory, and Martin 2010) at diagnosis is thought to be central to breast cancer and non-breast cancer morbidity (Demark-Wahnefried, Campbell, and Hayes 2012). This is currently being investigated further in a large ongoing trial (Rock et al. 2013). Adipocyte aromatase enzymes convert androgens to oestrogen derivatives, and when over expressed in the case of those with a higher number and larger adipocytes, may contribute to the proliferation of hormone sensitive cancers (Simpson et al. 1994, van Londen et al. 2011). However, it is not yet known if a reduction in adipose tissue creates a clinically significant decrease in aromatase enzymes to influence breast cancer outcomes. On the other hand, cardio-metabolic disease and subsequent mortality poses an equal or greater risk for survivors of breast cancer (Rock and Demark-Wahnefried 2002, Nichols et al. 2009). Furthermore, an increased waist girth and CRP are important risk factors in the development of metabolic syndrome in breast cancer survivors (Thomson et al. 2005, Healy et al. 2010).

LBM maintenance or increase in conjunction with moderate weight loss (1 to 3kg) is a clinically relevant outcome for women who have completed treatment for breast cancer (Ligibel and Goodwin 2012, Chlebowski et al. 2006). Our literature review summarised exercise, diet and combined modality interventions for during or after treatment for breast cancer. The synthesis of this information indicated that the best method to prevent loss of LBM and create a concurrent reduction in body fat is through combining structured exercise training and dietary prescription.

The magnitude of body weight loss experienced in the Ex+N-3 (2.27$\pm$1.9kg) is equal to that which was found to reduce breast cancer mortality over long term follow up (Chlebowski et al. 2006). A number of exercise interventions in breast cancer populations have produced decreases in waist girth (Guinan et al. 2013, Fernández-Lao et al. 2013). However, others have shown no effect for exercise (Irwin, Alvarez-Reeves, et al. 2009, Schmitz, Ahmed, et al. 2005) regardless of aerobic or resistance training prescription. In addition, energy restricted diets with (Harris et al. 2012, Scott et

al. 2013, Djuric 2011) or without (Thomson et al. 2010, Villarini et al. 2012) exercise prescription have induced waist reductions in breast cancer survivors.

LCn-3 supplementation has previously been associated with reductions in body weight (Kabir et al. 2007, Hill et al. 2007) and body fat% (Couet et al. 1997, Noreen et al. 2010, Munro and Garg 2012) in non-breast cancer populations. In contrast, no effect has been reported in other studies (Storlien et al. 2001, Kunesová et al. 2006, Krebs et al. 2006, Tierney et al. 2010, DeFina et al. 2011).

Mechanistic studies indicate that fatty oxidation may increase after both exercise and LCn-3 supplementation. Aerobic exercise has been shown to induce proliferation of mitochondria and up-regulation of β-oxidation enzymes (Holloway et al. 2006). While LCn-3 augment function of uncoupling protein-3 (Jaburek et al. 1999, Hun Cha et al. 2001) and fuel utilisation through the lipid catabolizing effects of peroxisome proliferator activated receptor (PPAR)-gamma (Flachs et al. 2009). This combination of factors give a theoretical basis on which to predict greater fatty acid oxidation in those exposed to both concurrently. Previously, Hill et al (2007) reported the effects on aerobic training with or without LCn-3 supplementation compared to sunflower oil placebo groups. An interaction of LCn-3 and exercise was not reported for body fat, however both LCn-3 and exercise were independently associated with decreases in body fat%.
Kabir et al (Kabir et al. 2007) noted that body fat mass and subcutaneous adipocyte diameter was decreased in the supplemented group compared to placebo, and Munro et al (2012) reported that body fat% change was associated with plasma DHA concentration. In addition, a large study examining the effects of high lean and fatty fish indicated a reduction in waist girth for men only, compared to a low seafood intake (Thorsdottir et al. 2007). In cancer populations undergoing chemotherapy, LCn-3 has been shown to reverse intramuscular triglyceride deposition over the course of treatment (Murphy et al. 2011).

In summary of finding relating to adiposty, our trial confirms evidence from in vitro and previous human studies that LCn-3 supplementation in combination with an exercise and nutrition program is associated with greater reductions in adiposity. No reductions in body weight, hip or waist girth were seen in either the LCn-3 supplementation or lifestyle program plus olive oil, indicating a potential synergistic effect for the combination of the two groups. Reduction of body fat% and waist circumference is an important consideration for breast cancer survivors who are at an increased risk of cardio-metabolic conditions. It is important to interpret these findings with caution, as body fat% and waist were not primary outcomes of the trial, however, our findings are relevant to practice.

A large intervention trial is currently under way to determine the effect of exercise and nutrition on long term breast cancer related outcomes, (Rock et al. 2013). While this study is not testing the effect of LCn-3, it may provide insight into the value of waist and body fat% reductions from a lifestyle program in a representative breast cancer population.

6.4.5 Muscle function and LBM change

Upper body strength increased significantly for the groups in the lifestyle program, while aerobic exercise and lower body strength was not changed at 24wks. LCn-3 was not observed to have an effect on strength-endurance or upper or lower body, nor on aerobic fitness. All groups improved handgrip strength and physical function (HAQ-DI) from baseline to 12wks. However, LCn-3 supplementation better maintained these improvements than those consuming olive oil.

Previous exercise intervention studies have reported improvements in cardiovascular fitness, muscle strength (Schmitz et al. 2009, Schmitz, Ahmed, et al. 2005) or both (Courneya et al. 2007, Herrero et al. 2006) regardless of change in body composition. Typically, strength increases of 20 to 65% (Schmitz et al. 2009, Schmitz, Ahmed, et al. 2005, Herrero et al. 2006) are noted after resistance training interventions, while increases in aerobic fitness have been consistently noted after aerobic exercise interventions (Schmitz et al. 2010). We noted significant increases in upper and lower body strength-endurance; upper body was only improved in those who participated in the lifestyle program. The push up test is a marker of upper body strength-endurance (American College of Sports Medicine 2010) and has been shown to increase when upper body resistance training is performed regularly (William et al. 2004). This improvement in push-ups performed is most likely due to the specificity of the training in the lifestyle program, i.e. push-ups were part of every supervised training session. This type of training may not be undertaken as commonly without prescription and supervision, such as in those who were given LCn-3 supplements only. Therefore, this may explain the comparatively lower improvement in the LCn-3 only group.

In terms of the effect of LCn-3 on muscle function, previous synergistic effects of LCn-3 and exercise training have been observed in middle-aged women (via reduced electromechanical delay) (Rodacki et al. 2012). The effect of LCn-3 on strength-endurance (i.e. number of push ups/squats in 1-minute) has not been tested previously. Thus, the understanding that LCn-3 may act through neural as opposed to through mechanical pathways may justify the lack of effect we noted for strength-endurance. However, we did observe better maintenance of maximum handgrip strength from 12 to 24wks for the LCn-3 supplemented groups independent of lifestyle program participation. Efficient neural activation is an important component of maximal force development (Fimland et al. 2009). In addition to this, evidence from dosing studies indicate that tissue

accumulation of LCn-3 occurs over two to six months (Yee et al. 2010). Thus it is plausible to hypothesise that a greater concentration of LCn-3 made available to the neural system by supplementation over 3 months, which in turn may have better maintained the ability to produce maximum force. Furthermore, these results agree with observations of a community dwelling population indicating a higher handgrip with fish consumption (Robinson et al. 2008), and compliment our finding that overall physical function followed the same pattern as handgrip strength.

Evidence from general populations indicate that function of LBM measured as muscle strength (Newman et al. 2006, Ruiz et al. 2008) or cardiorespiratory fitness (Lee, Blair, and Jackson 1999) is more predictive of mortality than LBM alone. Though, for the same fitness levels, greater total body weight is still associated with higher mortality (Hu et al. 2004). Similarly for breast cancer populations, stability or moderate decreases in weight are related to better survival (Caan et al. 2012, Caan et al. 2006, Kroenke et al. 2005, Chen et al. 2010). However, increasing physical activity to three or more hours per week of brisk walking is associated with 30-50% reductions in breast cancer mortality (Irwin et al. 2011, Ibrahim and Al-Homaidh 2010), and smaller but significant reductions in recurrence (Ibrahim and Al-Homaidh 2010). In other words, the function of the muscle, i.e. how it is being used, may be a predictor of outcomes for breast cancer survivors that is equally important to simple body weight, or body composition change.

6.5 Validity and feasibility of the intervention

First and foremost, our intervention was safe with there being no serious adverse events reported as result of the trial. The format of the lifestyle program was successfully carried out even though it faced similar challenges as would be faced in clinical practice, i.e. timing of sessions and availability of individuals within groups, and inconsistent group size due to changing rate of recruitment. In addition, as the groups progressed through the program, greater discussion, sharing of perspective and inter-participant motivation increased significantly. This component of the education sessions became as relevant and motivating as the pre-organised materials. The primary investigator noted that group cohesion made a difference to the overall atmosphere of the sessions (increased story sharing, humour, comrardery), and is an important consideration for future programs in practice.

The intervention could be replicated in a clinical setting at relatively low cost. Without including the cost of the clinician providing the services, all exercise equipment, stationery and blood results, was calculated at ~$300 per person. Of this, 75% was spent on blood analyses of LCn-3 (which

would not be necessary in practice) and exercise equipment (which was kept by each participant after the trial).

The home-based style exercise program was effective in that changes in strength were documented, however even with annotated video explanations of the exercises available to the participants, a significant amount of time was spent on technique correction during the supervised sessions. This reduced the intensity of these sessions, which formed 50% of the resistance training volume completed by the participants. Furthermore, motivation to complete the full exercise program at home was low, and this was reflected by a ~70% adherence to those exercises. As a suggestion, basing interventions of this type in a gym, and/or aligning with gyms and exercise physiologists in multiple locations is recommended. This would allow more supervised sessions to be conducted, and is also recommended to enhance safety and progression of the program.

In contrast, aerobic exercise increased significantly for all groups and was the preferred mode of exercise indicated by the above 100% adherence. Considering that mortality and recurrence rates are decreased by an increase in brisk walking (Ibrahim et al 2010), aerobic participation is a relevant outcome. However, it is noteworthy that both lifestyle program and supplementation only groups increased their physical activity.

Due to similar change in participation, dietary intake, and LBM, it is possible that just assessing body composition and providing basic information on physical activity and nutrition may be adequate to promote change in this population as a whole. As mentioned previously, our population may have been more inclined to participate in exercise as they expressed an interest in the trial initially. Thus, our participants may be more motivated than the general breast cancer population, from an activity/sedentary time perspective. However, considering the extensive assessment and follow-up treatment load placed on this population, recommending an intensive intervention may be over-burdening participants, and is contrary to a recent movement in ‘minimally disruptive care’ (Bohlen et al 2012). In contrast, certain individuals within the intervention thrived on the consistent follow-up, feedback and structured prescription offered by the weekly lifestyle program. Thus, rather than attempting to find the ‘optimal’ intervention, it may be more effective to offer a number of programs with varying intensity and involvement, then triaging patients on their preference, requirements and means.

LCn-3 and the lifestyle program combined were found to be more effective together than alone across a number of parameters, thus further research exploring diet-exercise-treatment synergies are

still important. Therefore, balancing the further research of program intensity and adherence, with synergistic interventions is needed to provide clinical programs a greater reach and impact.

Conclusions

Consumption of LCn-3s in combination with an exercise and nutrition program had a synergistic effect in reducing total body weight, waist girth and hip girth, while LBM was maintained. These findings agree with hypotheses generated from experimental evidence that LCn-3 and exercise lead to influence fat metabolism, and do so in a complementary manner. This is the first study to have reported this synergistic effect in humans, and the first trial to combine specific nutrients and exercise training in a population of women who have been diagnosed and treated for breast cancer. We found that motivation levels were sufficient to increase aerobic activity with or without structured prescription. However, only the addition of LCn-3 was associated with clinically significant weight loss, and body fat reductions, that have been associated with decreased mortality and decreased risk of metabolic disease in larger and longer trials.

Despite the recent evidence that LCn-3 may augment the response of LBM to exercise training, our study indicated that LCn-3, a 12-week exercise and nutrition lifestyle program, and a combination of the two all promote LBM maintenance without differences between interventions. This is most likely due to the fact that the LCn-3 only group significantly increased their level of activity in line with the lifestyle program groups. In addition, similar to the majority of research that reported little the effect of LCn-3 or exercise on CRP, we did not see a significant change for any group. Quality of life improved for all groups over the intervention period. This mirrors findings that quality of life improves generally in the year following treatment, which may also be related to the increased physical activity in all groups.

As expected from a wealth of prior research, structured and specific semi-supervised training improves upper body muscular strength-endurance. In contrast improvements in lower body strength-endurance were common to all groups independent of prescribed exercise. This is most likely due to the fact that the control group significantly increased their exercise volume from 12 to 24 weeks. In turn, this may indicate that current awareness of the importance of physical activity after breast cancer is adequate to motivate this population to engage in aerobic exercise. However, a similar engagement in resistance training was not seen in the LCn-3 only group.
Of interest, LCn-3 consumption was seen to promote better maintenance of grip strength, which was matched by an improved measure of daily physical function independent of structured exercise during the 12 to 24 week period. This agrees with the previous literature describing that increased tissue levels of LCn-3 are associated with greater grip strength and muscle function. Also, in agreement with our review, the delayed effect of LCn-3 supplementation on muscle function may

be due to the gradual accumulation of LCn-3 in the tissue over two to three months. Thus, prolonged supplementation may be key to elicit a significant change.

In all, these results indicate that LCn-3 is a useful nutrient in the prescription of nutrition for women who have completed treatment for breast cancer. Further research combining LCn-3 and exercise over a time period relevant to post-treatment cardio-metabolic outcomes is recommended to determine if these body composition changes convert to positive health outcomes.

Clinical implications

With increasing incidence of breast cancer diagnoses, and therefore an increased number of survivors at risk of recurrence and cardio metabolic conditions, efficacious interventions that positively affect body composition and post-treatment disease risk are of high importance.

Recommending high dose (3g/day of EPA plus DHA) LCn-3 in conjunction with a lifestyle program may clinically improve adiposity over and above that of LCn-3 or the lifestyle program in isolation.

Regular aerobic exercise is effective in preventing LBM loss after treatment for breast cancer. The addition of LCn-3 is likely to have little effect on this.

Prescription of an ad libitum healthy eating pattern, without specific energy restriction, is adequate to allow body weight and adiposity reductions without LBM decrease when paired with exercise and LCn-3 supplementation.

Prescription of high dose LCn-3 may improve physical function and maintain greater grip strength independently of exercise training.

Future Recommendations

Future recommendations for research in this area need to include outcomes that relate to disease-free survival, body composition with a focus on lean body mass and quality of life. This study has indicated that there is benefit for the combination of LCn-3 and exercise. Both interventions have been shown to have positive effects on long term health in breast cancer and non-breast cancer populations.

Enhancing disease-free life after breast cancer

The synergistic effects of exercise and nutrition prescription with LCn-3 supplementation on body weight, and waist and hip girths should be followed for a longer time period, such that changes in risk factors for cardio-metabolic disease and breast cancer related outcomes can be measured. Do the changes in body fat due to the dual intervention have a preventive effect for future disease?

Increasing LBM after breast cancer

In future studies aiming to increase LBM after treatment, a supervised and fully objectively monitored gym-based resistance and aerobic training program should be prescribed to enhance the intervention's effect on both muscular hypertrophy and adiposity reductions. At a higher intensity of training, conducted in a more accountable manner, dose of exercise in conjunction with LCn-3 may be elucidated.

To further assess the proposed effect of LCn-3 on LBM growth, it is suggested that it is combined with resistance training as described above, and validated through the measurement of intramuscular triglyceride (IMTG) concentration (MRI or CT scans). IMTG deposition in inactive individuals has been associated with insulin resistance and greater inflammation, and is increased after chemotherapy. Understanding the effects of LCn-3 on IMTG in conjunction with exercise training may provide a better understanding of metabolic pathways involved in the synergy. Additional, and potentially greater LBM growth may be possible through appropriate protein supplements in conjunction with structured supervised resistance training. Combining LCn-3, which seems to be permissive of LBM growth, with appropriately timed and sized protein meals may be more synergistic than the combination used in this study.

Quality of life after breast cancer

Measures of physical function (strength, cardiorespiratory fitness, performance of activities of daily living) should be included in future trials determining general outcomes for breast cancer survivors. If further evidence proves that measures of physical function are related to clinically relevant endpoints, it will allow exercise and nutrition prescription to target risk factors directly. Promoting behaviours to increase physical function rather than body composition may be more positive and may reduce the angst associated with increased adiposity.

References

Abete, I., D. Parra, A. B. Crujeiras, E. Goyenechea, and J. A. Martinez. 2008. "Specific insulin sensitivity and leptin responses to a nutritional treatment of obesity via a combination of energy restriction and fatty fish intake." *Journal of Human Nutrition and Dietetics* 21 (6):591-600.

Academy of Nutrition and Dietetics. 2010. *Academy of Nutrition and Dietetics Evidence Analysis Manual. CatN: 4297; ISBN: 978-0-88091-1*, *Research and Strategic Business Development*.

Alexander, Susanna, Ollie Minton, and Patrick C. Stone. 2009. "Evaluation of Screening Instruments for Cancer-Related Fatigue Syndrome in Breast Cancer Survivors." *J Clin Oncol* 27 (8):1197-1201. doi: 10.1200/jco.2008.19.1668.

Alfano, C. M., I. Imayama, M. L. Neuhouser, J. K. Kiecolt-Glaser, A. W. Smith, K. Meeske, A. McTiernan, L. Bernstein, K. B. Baumgartner, C. M. Ulrich, and R. Ballard-Barbash. 2012. "Fatigue, Inflammation, and omega-3 and omega-6 Fatty Acid Intake Among Breast Cancer Survivors." *Journal of Clinical Oncology* 30 (12):1280-1287. doi: 10.1200/jco.2011.36.4109.

American College of Sports, Medicine. 2009. "American College of Sports Medicine position stand. Progression models in resistance training for healthy adults." *Medicine and science in sports and exercise* 41 (3):687.

American College of Sports Medicine, ed. 2010. *ACSM's Guidelines for Exercise Testing and Prescription 8th Ed*: Wolters Kluwer/Lippincott Williams & Wilkins: Philadelphia.

Arsenault, Benoit J., Mélanie Côté, Amélie Cartier, Isabelle Lemieux, Jean-Pierre Després, Robert Ross, Conrad P. Earnest, Steven N. Blair, and Timothy S. Church. 2009. "Effect of exercise training on cardiometabolic risk markers among sedentary, but metabolically healthy overweight or obese post-menopausal women with elevated blood pressure." *Atherosclerosis* 207 (2):530-533. doi: 10.1016/j.atherosclerosis.2009.05.009.

Ash, S., M. Reeves, J. Bauer, T. Dover, A. Vivanti, C. Leong, T. O'Moore Sullivan, and S. Capra. 2006. "A randomised control trial comparing lifestyle groups, individual counselling and written information in the management of weight and health outcomes over 12 months." *Int J Obes* 30 (10):1557-1564.

Aslani, A., R. C. Smith, B. J. Allen, N. Pavlakis, and J. A. Levi. 1999. "Changes in body composition during breast cancer chemotherapy with the CMF-regimen." *Breast Cancer Research and Treatment* 57 (3):285-290.

Australian Institute of Health and Welfare & Cancer Australia. 2012. "Breast cancer in Australia: an overview." *Cancer series no. 71.* Cat. no. CAN 67. (Canberra: AIHW).

Basen-Engquist, Karen, Cindy L. Carmack Taylor, Carol Rosenblum, Murray A. Smith, Eileen H. Shinn, Anthony Greisinger, Xylina Gregg, Pamela Massey, Vicente Valero, and Edgardo Rivera. 2006. "Randomised pilot test of a lifestyle physical activity intervention for breast cancer survivors." *Patient Education and Counseling* 64 (1-3):225-234. doi: DOI: 10.1016/j.pec.2006.02.006.

Battaglini, C, M Bottaro, C Dennehy, L Rae, E Shields, D Kirk, and A Hackney. 2007. "The effects of an individualized exercise intervention on body composition in breast cancer patients undergoing treatment." *Sao Paulo Medical Journal* 125 (1):22-28.

Bauer, Jurgen, Gianni Biolo, Tommy Cederholm, Matteo Cesari, Alfonso J. Cruz-Jentoft, John Edward Morley, Stuart Phillips, Cornel Sieber, Peter Stehle, Daniel Teta, Renuka Visvanathan, Elena Volpi, and Yves Boirie. 2013. "Evidence-based recommendations for optimal dietary protein intake in older people: A position paper from the prot-age study group." *Journal of the American Medical Directors Association* 14 (8):542-559. doi: 10.1016/j.jamda.2013.05.021.

Belalcazar, L. Maria, Christie M. Ballantyne, Steven M. Haffner, Wei Lang, Ron C. Hoogeveen, Julia Rushing, Dawn C. Schwenke, Russell P. Tracy, F. Xavier Pi-Sunyer, Andrea M. Kriska, and Ahead Research Group Look. 2013. "Lifestyle intervention and/or statins for the reduction of C-reactive protein in type 2 diabetes: from the look AHEAD study." *Obesity (Silver Spring, Md.)* 21 (5):944-950. doi: 10.1002/oby.20431.

Beniamini, Y., J. J. Rubenstein, L. D. Zaichkowsky, and M. C. Crim. 1997. "Effects of high-intensity strength training on quality-of-life parameters in cardiac rehabilitation patients." *The American journal of cardiology* 80 (7):841-846. doi: 10.1016/S0002-9149(97)00533-X.

Berrino, Franco, Cristina Bellati, Giorgio Secreto, Edgarda Camerini, Valeria Pala, Salvatore Panico, Giovanni Allegro, and Rudolf Kaaks. 2001. "Reducing Bioavailable Sex Hormones through a Comprehensive Change in Diet: the Diet and Androgens (DIANA) Randomised Trial." *Cancer Epidemiology Biomarkers & Prevention* 10 (1):25-33.

Biglia, Nicoletta, Giulia Moggio, Elisa Peano, Paola Sgandurra, Riccardo Ponzone, Rossella E. Nappi, and Piero Sismondi. 2010. "Effects of surgical and adjuvant therapies for breast cancer on sexuality, cognitive functions, and body weight." *The journal of sexual medicine* 7 (5):1891. doi: 10.1111/j.1743-6109.2010.01725.x.

Bohlen, K., et al. (2012). "Overwhelmed Patients: A videographic analysis of how patients with type 2 diabetes and clinicians articulate and address treatment burden during clinical encounters." Diabetes care **35**(1): 47-49.

Boutros, Cherif, Ponnandai Somasundar, Anthony Razzak, Scott Helton, and N. Joseph Espat. 2010. "Omega-3 Fatty Acids: Investigations From Cytokine Regulation to Pancreatic Cancer Gene Suppression." *Arch Surg* 145 (6):515-520. doi: 10.1001/archsurg.2010.91.

Bower, Julienne E., Patricia A. Ganz, May Lin Tao, Wenhua Hu, Thomas R. Belin, Saviz Sepah, Steve Cole, and Najib Aziz. 2009. "Inflammatory biomarkers and fatigue during radiation therapy for breast and prostate cancer." *Clinical cancer research : an official journal of the American Association for Cancer Research* 15 (17):5534. doi: 10.1158/1078-0432.CCR-08-2584.

Brandt, C, and B K Pedersen. 2010. "The role of exercise-induced myokines in muscle homeostasis and the defense against chronic diseases." *Journal of Biomedicine and Biotechnology*. doi: 10.1155/2010/520258.

Breen, Leigh, and Stuart M. Phillips. 2013. "Interactions between exercise and nutrition to prevent muscle waste during ageing." *British journal of clinical pharmacology* 75 (3):708-715. doi: 10.1111/j.1365-2125.2012.04456.x.

Brown, Wendy J., Nicola W. Burton, Alison L. Marshall, and Yvette D. Miller. 2008. "Reliability and validity of a modified self-administered version of the Active Australia physical activity survey in a sample of mid-age women." *Australian and New Zealand Journal of Public Health* 32 (6):535-541.

Buckley, Jonathan D., and Peter R. C. Howe. 2010. "Long-Chain Omega-3 Polyunsaturated Fatty Acids May Be Beneficial for Reducing Obesity—A Review." *Nutrients* 2 (12):1212-1230.

Burd, Nicholas A., Daniel W. D. West, Aaron W. Staples, Philip J. Atherton, Jeff M. Baker, Daniel R. Moore, Andrew M. Holwerda, Gianni Parise, Michael J. Rennie, Steven K. Baker, and Stuart M. Phillips. 2010. "Low-Load High Volume Resistance Exercise Stimulates Muscle Protein Synthesis More Than High-Load Low Volume Resistance Exercise in Young Men." *PLoS ONE* 5 (8):e12033. doi: 10.1371/journal.pone.0012033.

Burnham, T R, and A Wilcox. 2002. "Effects of exercise on physiological and psychological variables in cancer survivors." *Medicine & Science in Sports & Exercise* 34 (12):1863-1867.

Caan, B J, A Emond, L Natarajan, A Castillo, E Gunderson, L Habel, and L Jones. 2006. "Post-diagnosis weight gain and breast cancer recurrence in women with early stage breast cancer." *Breast Cancer Research and Treatment* 99:47-57.

Caan, B J, M I Kwan, G Hartzell, A Castillo, and M Slattery. 2008. "Pre-diagnosis body mass index, post-diagnosis weight change, and prognosis among women with early stage breast cancer." *Cancer Causes Control* 19:1319-1328.

Caan, Bette J., Marilyn L. Kwan, Xiao Ou Shu, John P. Pierce, Ruth E. Patterson, Sarah J. Nechuta, Elizabeth M. Poole, Candyce H. Kroenke, Erin K. Weltzien, Shirley W. Flatt, Charles P.

Quesenberry, Michelle D. Holmes, and Wendy Y. Chen. 2012. "Weight Change and Survival after Breast Cancer in the After Breast Cancer Pooling Project." *Cancer Epidemiology Biomarkers & Prevention* 21 (8):1260-1271. doi: 10.1158/1055-9965.epi-12-0306.

Calder, P C. 2012. "Omega-3 polyunsaturated fatty acids and inflammatory processes: nutrition or pharmacology?" *British Journal of Nutrition* 75 (3):645-662. doi: 10.1111/j.1365-2125.2012.04374.x.

Camoriano, J. K., C. L. Loprinzi, J. N. Ingle, T. M. Therneau, J. E. Krook, and M. H. Veeder. 1990. "Weight change in women treated with adjuvant therapy or observed following mastectomy for node-positive breast cancer." *Journal of Clinical Oncology* 8 (8):1327-1334.

Cardiel, M. H., et al. (1993). "How to measure health status in rheumatoid arthritis in non-English speaking patients: validation of a Spanish version of the Health Assessment Questionnaire Disability Index (Spanish HAQ-DI)." Clinical Experimental Rheumatology **11**(2): 117-121

Cerchietti, L C A , A H Naviganteac, and M A Castroa. 2007. "Effects of Eicosapentaenoic and Docosahexaenoic n-3 Fatty Acids From Fish Oil and Preferential Cox-2 Inhibition on Systemic Syndromes in Patients With Advanced Lung Cancer " *Nutrition and Cancer* 59 (1):14-20. doi: 10.1080/01635580701365068

Cella, D., et al. (2002). "Combining Anchor and Distribution-Based Methods to Derive Minimal Clinically Important Differences on the Functional Assessment of Cancer Therapy (FACT) Anemia and Fatigue Scales." Journal of pain and symptom management **24**(6): 547-561.

Chad, Karen E., Bruce A. Reeder, Elizabeth L. Harrison, Nigel L. Ashworth, Suzanne M. Sheppard, Sandra L. Schultz, Brenda G. Bruner, Koren L. Fisher, and Joshua A. Lawson. 2005. "Profile of physical activity levels in community-dwelling older adults." *Medicine and science in sports and exercise* 37 (10):1774-1784. doi: 10.1249/01.mss.0000181303.51937.9c.

Chan, Dick C., Gerald F. Watts, P. Hugh R. Barrett, Lawrence J. Beilin, and Trevor A. Mori. 2002. "Effect of Atorvastatin and Fish Oil on Plasma High-Sensitivity C-Reactive Protein Concentrations in Individuals with Visceral Obesity." *Clinical Chemistry* 48 (6):877.

Chapkin, R S, W Kim, J R Lupton, and D N McMurray. 2009. "Dietary docosahexaenoic and eicosapentaenoic acid: Emerging mediators of inflammation." *Prostaglandins, Leukotrienes and Essential Fatty Acids* 81:187-191.

Chattha, Ritu, Nagarathna Raghuram, Padmalatha Venkatram, and Nagendra R. Hongasandra. 2008. "Treating the climacteric symptoms in Indian women with an integrated approach to yoga therapy: a randomised control study." *Menopause (New York, N.Y.)* 15 (5):862-870. doi: 10.1097/gme.0b013e318167b902.

Chen, Xiaoli, Wei Lu, Wei Zheng, Kai Gu, Zhi Chen, Ying Zheng, and Xiao Shu. 2010. "Obesity and weight change in relation to breast cancer survival." *Breast Cancer Research and Treatment* 122 (3):823-888. doi: 10.1007/s10549-009-0708-3.

Cheney, C L, J Mahloch, and P Freeny. 1994. "Computerised tomography assessment of women with weight changes associated with adjuvant treatment for breast cancer." *American Journal of Clinical Nutrition* 66:141-146.

Chlebowski, Rowan T., George L. Blackburn, Cynthia A. Thomson, Daniel W. Nixon, Alice Shapiro, M. Katherine Hoy, Marc T. Goodman, Armando E. Giuliano, Njeri Karanja, Philomena McAndrew, Clifford Hudis, John Butler, Douglas Merkel, Alan Kristal, Bette Caan, Richard Michaelson, Vincent Vinciguerra, Salvatore Del Prete, Marion Winkler, Rayna Hall, Michael Simon, Barbara L. Winters, and Robert M. Elashoff. 2006. "Dietary Fat Reduction and Breast Cancer Outcome: Interim Efficacy Results From the Women's Intervention Nutrition Study." *J. Natl. Cancer Inst.* 98 (24):1767-1776. doi: 10.1093/jnci/djj494.

Connolly, B S, C Barnett, K N Vogt, T Li, J Stone, and N F Boyd. 2002. "A Meta-Analysis of Published Literature on Waist-to-Hip Ratio and Risk of Breast Cancer." *Nutrition and Cancer* 44 (2):127-138.

Cormie, Prue, Daniel A. Galvão, Nigel Spry, and Robert U. Newton. 2013. "Neither Heavy nor Light Load Resistance Exercise Acutely Exacerbates Lymphedema in Breast Cancer Survivor." *Integrative Cancer Therapies* 12 (5):423-432. doi: 10.1177/1534735413477194.

Couet, C, J Delarue, P Ritz, J-M Antoine, and F Lamisse. 1997. "Effect of dietary fish oil on body fat mass and basal fat oxidation in healthy adults." *International Journal of Obesity* 21:637-643.

Courneya, Kerry S., Christine M. Friedenreich, Rami A. Sela, H. Arthur Quinney, Ryan. E. Rhodes, and Michael Handman. 2003. "The group psychotherapy and home-based physical exercise (group-hope) trial in cancer survivors: Physical fitness and quality of life outcomes." *Psycho-Oncology* 12 (4):357-374.

Courneya, Kerry S., John R. Mackey, Gordon J. Bell, Lee W. Jones, Catherine J. Field, and Adrian S. Fairey. 2003. "Randomised Controlled Trial of Exercise Training in Postmenopausal Breast Cancer Survivors: Cardiopulmonary and Quality of Life Outcomes." *J Clin Oncol* 21 (9):1660-1668. doi: 10.1200/jco.2003.04.093.

Courneya, Kerry S., Roanne J. Segal, John R. Mackey, Karen Gelmon, Robert D. Reid, Christine M. Friedenreich, Aliya B. Ladha, Caroline Proulx, Jeffrey K.H. Vallance, Kirstin Lane, Yutaka Yasui, and Donald C. McKenzie. 2007. "Effects of Aerobic and Resistance Exercise

in Breast Cancer Patients Receiving Adjuvant Chemotherapy: A Multicenter Randomised Controlled Trial." *J Clin Oncol* 25 (28):4396-4404. doi: 10.1200/jco.2006.08.2024.

Crochemore, Izabella Candido Carvalho, Aline F. P. Souza, Andressa C. F. de Souza, and Eliane Lopes Rosado. 2012. "ω-3 Polyunsaturated Fatty Acid Supplementation Does Not Influence Body Composition, Insulin Resistance, and Lipemia in Women With Type 2 Diabetes and Obesity." *Nutrition in Clinical Practice* 27 (4):553-60. doi: 10.1177/0884533612444535.

Dale, Caroline E., Ann Bowling, Joy Adamson, Hannah Kuper, Antoinette Amuzu, Shah Ebrahim, Juan P. Casas, and Eveline Nüesch. 2013. "Predictors of patterns of change in health-related quality of life in older women over 7 years: evidence from a prospective cohort study." *Age and ageing* 42 (3):312.

Daley, Amanda J., Helen Crank, John M. Saxton, Nanette Mutrie, Robert Coleman, and Andrea Roalfe. 2007. "Randomised Trial of Exercise Therapy in Women Treated for Breast Cancer." *J Clin Oncol* 25 (13):1713-1721. doi: 10.1200/jco.2006.09.5083.

Daley, Amanda J., Nanette Mutrie, Helen Crank, Robert Coleman, and John Saxton. 2004. "Exercise therapy in women who have had breast cancer: design of the Sheffield women's exercise and well-being project." *Health Educ. Res.* 19 (6):686-697. doi: 10.1093/her/cyg094.

Daling, J. R., K. E. Malone, D. R. Doody, L. G. Johnson, J. R. Gralow, and P. L. Porter. 2001. "Relation of body mass index to tumor markers and survival among young women with invasive ductal breast carcinoma." *Cancer* 92:720-729. doi: 10.1002/1097-0142.

Daly, Robin M, Stella L O'Connell, Niamh L Mundell, Carley A Grimes, David W. Dunstan, and Caryl A Nowson. 2012. "A protein-rich diet utilizing lean red meat in combination with progressive resistance training reduces inflammation and enhances muscle mass and strength in elderly women " *International Congress of Dietetics - Conference Poster*. doi: http://www.emobilise.com.au/uploads/1209015abstract00842.pdf.

de Lorgeril, M and Salen, P. 2014 "Helping women to good health: breast cancer, omega-3/omega-6 lipids, and related lifestyle factors." *BMC Medicine* 12:54. doi: 10.1186/1741-7015-12-54

de Waard, F, R Ramlau, Y Mulders, T de Vries, and S van Waveren. 1993. "A feasibility study on weight reduction in obese postmenopausal breast cancer patients." *European Journal of Cancer Prevention* 2 (3):233-238.

Debigare, R, C H Cote, and F Maltais. 2001. "Peripheral muscle wast- ing in chronic obstructive pulmonary disease." *American Journal of Respiratory and Critical Care Medicine* 164:1712-1717.

Dee, Anne. 2010. "DEXA measures of body fat percentage and acute phase proteins among breast cancer survivors." *University of Southern California*. doi: AAT 1476139.

Dee, Anne, Leslie Bernstein, Roberta McKean-Cowdin, Marian L. Neuhouser, Cornelia Ulrich, Richard N. Baumgartner, Anne McTiernan, Kathy Baumgartner, Catherine M. Alfano, and Rachel Ballard-Barbash. 2012. "DEXA measures of body fat percentage and acute phase proteins among breast cancer survivors: a cross-sectional analysis." *BMC cancer* 12 (1):343-343. doi: 10.1186/1471-2407-12-343.

DeFina, Laura F, Lucille G Marcoux, Susan M Devers, Joseph P Cleaver, and Benjamin L Willis. 2011. "Effects of omega-3 supplementation in combination with diet and exercise on weight loss and body composition." *The American Journal of Clinical Nutrition* 93 (2):455-462. doi: 10.3945/ajcn.110.002741.

Demark-Wahnefried, W , B L Peterson, E P Winer, L Marks, N Aziz, P K Marcom, K Blackwell, and B K Rimer. 2001. "Changes in Weight, Body Composition, and Factors Influencing Energy Balance Among Premenopausal Breast Cancer Patients Receiving Adjuvant Chemotherapy." *Journal of Clinical Oncology* 19:2381-2389.

Demark-Wahnefried, W, V Hars, M R Conaway, K Havlin, B K Rimer, G McElveen, and E P Winer. 1997. "Reduced rates of metabolism and decreased physical activity in breast cancer patients receiving adjuvant chemotherapy." *American Journal of Clinical Nutrition* 65 (5):1495-1501.

Demark-Wahnefried, W, A J Kenyon, P Eberle, A Skye, and W Kraus. 2002. "Preventing sarcopenic obesity among breast cancer patients who receive adjuvant chemotherapy: results of a feasibility study." *Clinical Exercise Physiology* 4 (1):44-49.

Demark-Wahnefried, Wendy, Kristin L. Campbell, and Sandra C. Hayes. 2012. "Weight management and its role in breast cancer rehabilitation." *Cancer* 118 (8 Suppl):2277-2287. doi: 10.1002/cncr.27466.

Demark-Wahnefried, Wendy, L. Douglas Case, Kimberly Blackwell, P. Kelly Marcom, William Kraus, Noreen Aziz, Denise Clutter Snyder, Jeffrey Giguere, and Edward Shaw. 2008. "Results of a Diet/Exercise Feasibility Trial to Prevent Adverse Body Composition Change in Breast Cancer Patients on Adjuvant Chemotherapy." *Clinical Breast Cancer* 8 (1):70-79.

Demark-Wahnefried, Wendy, Elizabeth C. Clipp, Isaac M. Lipkus, David Lobach, Denise Clutter Snyder, Richard Sloane, Bercedis Peterson, Jennifer M. Macri, Cheryl L. Rock, Colleen M. McBride, and William E. Kraus. 2007. "Main Outcomes of the FRESH START Trial: A Sequentially Tailored, Diet and Exercise Mailed Print Intervention Among Breast and Prostate Cancer Survivors." *J Clin Oncol* 25 (19):2709-2718. doi: 10.1200/jco.2007.10.7094.

Demark-Wahnefried, Wendy, Kristin L. Campbell, and Sandra C. Hayes. 2012. "Weight management and its role in breast cancer rehabilitation." *Cancer* 118 (S8):2277-2287. doi: 10.1002/cncr.27466.

DeNysschen, C A , J K Brown, M H Cho, and M J Dodd. 2011. "Nutritional Symptom and Body Composition Outcomes of Aerobic Exercise in Women With Breast Cancer." *Clinical Nursing Research* 20 (1):29-46.

Din, Omar, David Dodwell, Richard Wakefield, and Robert Coleman. 2010. "Aromatase inhibitor-induced arthralgia in early breast cancer: what do we know and how can we find out more?" *Breast Cancer Research and Treatment* 120 (3):525-538.

DiSipio, T., et al. (2013). "Incidence of unilateral arm lymphoedema after breast cancer: a systematic review and meta-analysis." The Lancet Oncology **14**(6): 500-515.

Djuric, Zora. 2011. "A Diet and Exercise Intervention during Chemotherapy for Breast Cancer." *The Open Obesity Journal* 3 (1):87-97. doi: 10.2174/1876823701103010087.

Djuric, Zora, Nora M. DiLaura, Isabella Jenkins, Linda Darga, Catherine K. L. Jen, Darlene Mood, Ellen Bradley, and William M. Hryniuk. 2002. "Combining Weight-Loss Counseling with the Weight Watchers Plan for Obese Breast Cancer Survivors." *Obesity* 10 (7):657-665.

Dunstan, D W, E L M Barr, G N Healy, J Salmon, J E Shaw, B Balkau, D J Magliano, A J Cameron, P Z Zimmet, and N Owen. 2010. "Television Viewing Time and Mortality." *Circulation* 121:384-391.

Easter, Linda, Melanie J. Bopp, Stephen B. Kritchevsky, Denise K. Houston, Barbara J. Nicklas, and Leon Lenchik. 2008. "Lean mass loss is associated with low protein intake during dietary-induced weight loss in postmenopausal women." *Journal of the American Dietetic Association* 108 (7):1216.

Egger, Garry, and John Dixon. 2009. "Obesity and chronic disease: always offender or often just accomplice?" *British Journal of Nutrition* 102 (8):1238-1242. doi: 10.1017/S0007114509371676.

Esposito, K, A Pontillo, C Di Palo, G Giugliano, M Masella, R Marfella, and D Giugliano. 2003. "Effect of Weight Loss and Lifestyle Changes on Vascular Inflammatory Markers in Obese Women." *Journal of the American Medical Association* 289 (14):1799-1804.

Ewertz, Marianne, Maj-Britt Jensen, Katrín Á Gunnarsdóttir, Inger Højris, Erik H. Jakobsen, Dorte Nielsen, Lars E. Stenbygaard, Ulla B. Tange, and Søren Cold. 2011. "Effect of obesity on prognosis after early-stage breast cancer." *Journal of clinical oncology : official journal of the American Society of Clinical Oncology* 29 (1):25-31. doi: 10.1200/jco.2010.29.7614.

Fairey, Adrian S, K S Courneya, Catherine J. Field, Gordon J. Bell, Beth Jones, B St. Martin, and B E Mackey. 2005. "Effect of exercise training on C-reactive protein in postmenopausal

breast cancer survivors: A randomised controlled trial." *Brain, Behaviour, and Immunity* 19:381-388.

Fanin, Marina, Stefania Caira, Carlo Ori, Corrado Angelini, Carlo Riccardo Rossi, and Domenico Marco Bonifati. 2000. "Neuromuscular damage after hyperthermic isolated limb perfusion in patients with melanoma or sarcoma treated with chemotherapeutic agents." *Cancer Chemotherapy and Pharmacology* 46 (6):517-522.

Farzaneh-Far, Ramin, William S. Harris, Sachin Garg, Beeya Na, and Mary A. Whooley. 2009. "Inverse association of erythrocyte n-3 fatty acid levels with inflammatory biomarkers in patients with stable coronary artery disease: The Heart and Soul Study." *Atherosclerosis* 205 (2):538-543. doi: 10.1016/j.atherosclerosis.2008.12.013.

Fearon, K C H, W. J. Evans, and S. D. Anker. 2011. "Myopenia—a new universal term for muscle wasting " *Journal of Cachexia, Sarcopenia and Muscle* 2011 (2):1-3. doi: 10.1007/s13539-011-0025-7.

Fearon, K C H, M F von Meyenfeldt, A G W Moses, R van Geenen, A Roy, D J Gouma, A Giacosa, A Van Gossum, J Bauer, M D Barber, N K Aaronson, A C Voss, and M J Tisdale. 2003. "Effect of a protein and energy dense n-3 fatty acid enriched oral supplement on loss of weight and lean tissue in cancer cachexia: a randomised double blind trial." *Gut* 52 (10):1479-1486. doi: 10.1136/gut.52.10.1479.

Fearon, Kenneth C.H., Matthew D. Barber, Alastair G. Moses, Sam H. Ahmedzai, Gillian S. Taylor, Michael J. Tisdale, and Gordon D. Murray. 2006. "Double-Blind, Placebo-Controlled, Randomised Study of Eicosapentaenoic Acid Diester in Patients With Cancer Cachexia." *Journal of Clinical Oncology* 24 (21):3401-3407. doi: 10.1200/jco.2005.04.5724.

Ferlay, J , H R Shin, F Bray, D Forman, C Mathers, and D M Parkin. 2010. "Cancer Incidence and Mortality Worldwide: IARC CancerBase No. 10." GLOBOCAN Accessed May. Available from: http://globocan.iarc.fr.

Fernández-Lao, Carolina, Irene Cantarero-Villanueva, Angelica Ariza-Garcia, Carol Courtney, César Fernández-de-las-Peñas, and Manuel Arroyo-Morales. 2013. "Water versus land-based multimodal exercise program effects on body composition in breast cancer survivors: a controlled clinical trial." *Supportive Care in Cancer* 21 (2):521-530. doi: 10.1007/s00520-012-1549-x.

Festa, A., D'Agostino, R., Williams, K., Karter, A. J., Mayer-Davis, E. J., Tracy, R. P., Haffner, S. M. (2001). "The relation of body fat mass and distribution to markers of chronic inflammation." International journal of obesity and related metabolic disorders : journal of the International Association for the Study of Obesity **25**(10): 1407-1415

Fetterman Jr, James W., and Martin M. Zdanowicz. 2009. "Therapeutic potential of n-3 polyunsaturated fatty acids in disease." *American Journal of Health-System Pharmacy* 66 (13):1169-1179.

Fields, D. A., Goran, M., McCrory, M. A. (2002). "Body-composition assessment via air-displacement plethysmography in adults and children: a review." The American Journal of Clinical Nutrition **75**(3): 453

Fimland, Marius S., Jan Helgerud, Markus Gruber, Gunnar Leivseth, and Jan Hoff. 2009. "Functional maximal strength training induces neural transfer to single-joint tasks." *European journal of applied physiology* 107 (1):21-29. doi: 10.1007/s00421-009-1096-4.

Flachs, P., O. Horakova, P. Brauner, M. Rossmeisl, P. Pecina, N. Franssen-van Hal, J. Ruzickova, J. Sponarova, Z. Drahota, C. Vlcek, J. Keijer, J. Houstek, and J. Kopecky. 2005. "Polyunsaturated fatty acids of marine origin upregulate mitochondrial biogenesis and induce β-oxidation in white fat." *Diabetologia* 48 (11):2365-2375. doi: 10.1007/s00125-005-1944-7.

Flachs, Pavel, Martin Rossmeisl, Morten Bryhn, and Jan Kopecky. 2009. "Cellular and molecular effects of n−3 polyunsaturated fatty acids on adipose tissue biology and metabolism." *Clinical Science* 116 (1):1-16. doi: 10.1042/cs20070456.

Flynn, Mary M., and Steven E. Reinert. 2010. "Comparing an olive oil-enriched diet to a standard lower-fat diet for weight loss in breast cancer survivors: a pilot study." *Journal of women's health* 19 (6):1155-1161.

Flynn, Michael G., Brian K. McFarlin, and Melissa M. Markofski. 2007. "State of the Art Reviews: The Anti-Inflammatory Actions of Exercise Training." *American Journal of Lifestyle Medicine* 1 (3):220-235. doi: 10.1177/1559827607300283.

Forbes, G B, M R Brown, S L Welle, and B A Lipinski. 1996. "Deliberate overfeeding in women and men: energy cost and composition of the weight gain." *British Journal of Nutrition* 56:1-9.

Francini, G, R Petrioli, A Montagnani, A Cadirni, S Campagna, E Francini, and S Gonnelli. 2006. "Exemestane after tamoxifen as adjuvant hormonal therapy in postmenopausal women with breast cancer: effects on body composition and lipids." *British Journal of Cancer* 95:153-158.

Gadéa, E., et al. (2011). "Importance of metabolic changes induced by chemotherapy on prognosis of early-stage breast cancer patients: a review of potential mechanisms." Obesity Reviews **13**: 368-380.

Gau, Gerald , Ray W Squires, Thomas G Allison, Randal J Thomas, Iftikhar Kullo, and Stephen L Kopecky. 2010. "Cardiopulmonary fitness is associated with lower cardiovascular

mortality in a community based cohort." *Journal of the American College of Cardiology* 55 (10):A59.

Goodwin, P J, M Ennis, K I Pritchard, D McCready, J Koo, S Sidlofsky, M Trudeau, N Hood, and S Redwood. 1999. "Adjuvant Treatment and Onset of Menopause Predict Weight Gain After Breast Cancer Diagnosis." *Journal of Clinical Oncology* 17:120-129.

Goodwin, P J, M Ennis, K I Pritchard, M E Trudeau, J Koo, and Y Madarnas. 2002. "Fasting insulin and outcome in early stage breast cancer: results of a prospective cohort study." *Journal of Clinical Oncology* 20 (1):42-51.

Greene, J G. 1998. "Constructing a standard climacteric scale." *Maturitas* 29:25-31.

Guinan, E., J. Hussey, J. M. Broderick, F. E. Lithander, D. O'Donnell, M. J. Kennedy, and E. M. Connolly. 2013. "The effect of aerobic exercise on metabolic and inflammatory markers in breast cancer survivors-a pilot study." *Supportive care in cancer* 21 (7):1983-1992. doi: 10.1007/s00520-013-1743-5.

Haffner, Steven, Robert Ratner, Elizabeth Barrett-Connor, Marinella Temprosa, Jill Crandall, Sarah Fowler, Ronald Goldberg, Edward Horton, Santica Marcovina, Kieren Mather, Trevor Orchard, Group The Diabetes Prevention Program Research, and Group Diabetes Prevention Program Research. 2005. "Intensive Lifestyle Intervention or Metformin on Inflammation and Coagulation in Participants With Impaired Glucose Tolerance." *Diabetes* 54 (5):1566-1572. doi: 10.2337/diabetes.54.5.1566.

Hamilton, Marc T., Deborah G. Hamilton, and Theodore W. Zderic. 2007. "Role of Low Energy Expenditure and Sitting in Obesity, Metabolic Syndrome, Type 2 Diabetes, and Cardiovascular Disease." *Diabetes* 56 (11):2655-2667. doi: 10.2337/db07-0882.

Hanrahan, Emer O., Ana M. Gonzalez-Angulo, Sharon H. Giordano, Roman Rouzier, Kristine R. Broglio, Gabriel N. Hortobagyi, and Vicente Valero. 2007. "Overall survival and cause-specific mortality of patients with stage T1a,bN0M0 breast carcinoma." *Journal of clinical oncology : official journal of the American Society of Clinical Oncology* 25 (31):4952-4960. doi: 10.1200/JCO.2006.08.0499.

Harris, H. R., L. Bergkvist, and A. Wolk. 2012. "Alcohol intake and mortality among women with invasive breast cancer." *Br J Cancer* 106 (3):592-595.

Harris, Melissa Nauta, Damon L Swift, Valerie H Myers, Conrad P Earnest, Neil M Johannsen, Catherine M Champagne, Becky D Parker, Erma Levy, Katherine C Cash, and Timothy S Church. 2012. "Cancer Survival Through Lifestyle Change (CASTLE): a Pilot Study of Weight Loss." *International Journal of Behavioral Medicine*:1-10. doi: 10.1007/s12529-012-9234-5.

Harvie, M., L. Hooper, and A. H. Howell. 2003. "Central obesity and breast cancer risk: a systematic review." *Obesity Reviews* 4 (3):157-173. doi: 10.1046/j.1467-789X.2003.00108.x.

Harvie, Michelle. 2010. "The Importance of Controlling Body Weight After a Diagnosis of Breast Cancer: The Role of Diet and Exercise in Breast Cancer Patient Management." In *Exercise and Cancer Survivorship*, edited by Amanda Daley John Saxton, 73-96. Springer New York.

Harvie, Michelle N., I.T. Campbell, A. Baildam, and A. Howell. 2004. "Energy Balance in Early Breast Cancer Patients Receiving Adjuvant Chemotherapy." *Breast Cancer Research and Treatment* 83 (3):201-210.

Hayes, S. C., et al. (2008). "Lymphedema After Breast Cancer: Incidence, Risk Factors, and Effect on Upper Body Function." Journal of Clinical Oncology **26**(21): 3536-3542.

Hayes, S C, R R Spence, D A Galvao, and R U Newton. 2009. "Australian Association for Exercise and Sport Science position stand: Optimising cancer outcomes through exercise." *Journal of Science and Medicine in Sport* 12:428-434.

Hayes, Sandra C., Sheree Rye, Tracey DiSipio, Patsy Yates, John Bashford, Chris Pyke, Christobel Saunders, Diana Battistutta, and Elizabeth Eakin. 2013. "Exercise for health: a randomised, controlled trial evaluating the impact of a pragmatic, translational exercise intervention on the quality of life, function and treatment-related side effects following breast cancer." *Breast Cancer Research and Treatment* 137 (1):175-186. doi: 10.1007/s10549-012-2331-y.

Healy, Genevieve N., Charles E. Matthews, David W. Dunstan, Elisabeth A. H. Winkler, and Neville Owen. 2011. "Sedentary time and cardio-metabolic biomarkers in US adults: NHANES 2003–06." *European Heart Journal* 32 (5):590-7. doi: 10.1093/eurheartj/ehq451.

Healy, L.A., A.M. Ryan, P. Carroll, D. Ennis, V. Crowley, T. Boyle, M.J. Kennedy, E. Connolly, and J.V. Reynolds. 2010. "Metabolic Syndrome, Central Obesity and Insulin Resistance are Associated with Adverse Pathological Features in Postmenopausal Breast Cancer." *Clinical Oncology* 22 (4):281-288. doi: 10.1016/j.clon.2010.02.001.

Hebert, James R., Cara B. Ebbeling, Barbara C. Olendzki, Thomas G. Hurley, Yunsheng Ma, Nancy Saal, Judith K. Ockene, and Lynn Clemow. 2001. "Change in Women's Diet and Body Mass Following Intensive Intervention for Early-stage Breast Cancer." *Journal of the American Dietetic Association* 101 (4):421-431. doi: Doi: 10.1016/s0002-8223(01)00109-2.

Healy, Genevieve N. Matthews, Charles E. Dunstan, David W.Winkler, Elisabeth A. H., Owen, Neville (2011). "Sedentary time and cardio-metabolic biomarkers in US adults: NHANES 2003–06." European Heart Journal **32**(5): 590-597

Heesch, Kristiann C., Jannique G. Z. van Uffelen, Yolanda R. van Gellecum, and Wendy J. Brown. 2012. "Dose-response relationships between physical activity, walking and health-related quality of life in mid-age and older women." *Journal of epidemiology and community health* 66 (8):670.

Heiss, C. J., et al. (2008). "A Small Food or Fluid Load Has No Effect on Body Composition Measured by 3 Different Methods." Topics in Clinical Nutrition **23**(3): 229-233

Herrero, F, A F San Juan, S J Fleck, J Balmer, M Pérez, S Cañete, C P Earnest, C Foster, and A Lucía. 2006. "Combined Aerobic and Resistance Training in Breast Cancer Survivors: A Randomised, Controlled Pilot Trial." *International Journal of Sports Medicine* 27 (7):573-580.

Herrero, F., A. F. San Juan, S. J. Fleck, C. Foster, and A. Lucia. 2007. "Effects of Detraining on the Functional Capacity of Previously Trained Breast Cancer Survivors." *Int J Sports Med* 28 (03):257-264.

Hickey, M., C. Saunders, A. Partridge, N. Santoro, H. Joffe, and V. Stearns. 2008. "Practical clinical guidelines for assessing and managing menopausal symptoms after breast cancer." *Annals of Oncology* 19 (10):1669-1680. doi: 10.1093/annonc/mdn353.

Hill, A, J D Buckley, K J Murphy, and P R C Howe. 2007. "Combining fish-oil supplements with regular aerobic exercise improves body composition and cardiovascular disease risk factors." *American Journal of Clinical Nutrition* 85:1267-1274.

Hlavaty, P, M Kunesova, M Gojova, E Tvrzicka, M Vecka, P Roubal, M Hill, K Hlavata, P Kalouskova, V Hainer, A Zak, and J Drbohlav. 2008. "Change in Fatty Acid Composition of Serum Lipids in Obese Females After Short-Term Weight-reducing Regimen with the Addition of n-3 Long Chain Polyunsaturated Fatty Acids in Comparison to Controls." *Physiological Research* 57 (Supp 1):S57-S65.

Hogg, R J, L Fitzgibbons, C Atkins, N Nardelli, and R C Bay. 2006. "Efficacy of Omega-3 Fatty Acids in Children and Adults with IgA Nephropathy Is Dosage and Size-Dependent." *Clinical Journal of the American Society of Nephrology* 1:1167-1172.

Holloway, Graham P., Veronic Bezaire, George J. F. Heigenhauser, Narendra N. Tandon, Jan F. C. Glatz, Joost J. F. P. Luiken, Arend Bonen, and Lawrence L. Spriet. 2006. "Mitochondrial long chain fatty acid oxidation, fatty acid translocase/CD36 content and carnitine palmitoyltransferase I activity in human skeletal muscle during aerobic exercise." *The Journal of Physiology* 571 (1):201-210. doi: 10.1113/jphysiol.2005.102178.

Howard-Anderson, Jessica, Patricia A. Ganz, Julienne E. Bower, and Annette L. Stanton. 2012. "Quality of Life, Fertility Concerns, and Behavioral Health Outcomes in Younger Breast

Cancer Survivors: A Systematic Review." *Journal of the National Cancer Institute* 104 (5):386-405. doi: 10.1093/jnci/djr541.

Hsu, Tina, Marguerite Ennis, Nicky Hood, Margaret Graham, and Pamela J. Goodwin. 2013. "Quality of Life in Long-Term Breast Cancer Survivors." *Journal of Clinical Oncology* 31 (28):3540-3548. doi: 10.1200/jco.2012.48.1903.

Hu, Frank B., Walter C. Willett, Tricia Li, Meir J. Stampfer, Graham A. Colditz, and JoAnn E. Manson. 2004. "Adiposity as Compared with Physical Activity in Predicting Mortality among Women." *The New England Journal of Medicine* 351 (26):2694-2703. doi: 10.1056/NEJMoa042135.

Hubal, Monica J., Paul S. Visich, Robert F. Zoeller, Richard L. Seip, Priscilla M. Clarkson, Heather Gordish-Dressman, Paul D. Thompson, Thomas B. Price, Eric P. Hoffman, Theodore J. Angelopoulos, Paul M. Gordon, Niall M. Moyna, and Linda S. Pescatello. 2005. "Variability in muscle size and strength gain after unilateral resistance training." *Medicine and science in sports and exercise* 37 (6):964.

Hun Cha, Seung, Akiko Fukushima, Keiko Sakuma, and Yasuo Kagawa. 2001. "Chronic Docosahexaenoic Acid Intake Enhances Expression of the Gene for Uncoupling Protein 3 and Affects Pleiotropic mRNA Levels in Skeletal Muscle of Aged C57BL/6NJcl Mice." *Journal of Nutrition* 131 (1):2636-2642.

Hutchins-Wiese, H. L., A. Kleppinger, K. Annis, E. Liva, C. J. Lammi-Keefe, H. A. Durham, and A. M. Kenny. 2013. "The impact of supplemental n-3 long chain polyunsaturated fatty acids and dietary antioxidants on physical performance in postmenopausal women." *The journal of nutrition, health & aging* 17 (1):76-80. doi: 10.1007/s12603-012-0415-3.

Ibrahim, Ezzeldin, and Abdelaziz Al-Homaidh. 2010. "Physical activity and survival after breast cancer diagnosis: meta-analysis of published studies." *Medical Oncology* 28 (3):753 - 765. doi: 10.1007/s12032-010-9536-x.

Ingram, Carolyn, and J K Brown. 2004. "Patterns of weight and body composition change in premenopausal women with early stage breast cancer." *Cancer Nursing* 27 (6):483-490.

Ingram, Carolyn, Kerry S. Courneya, and Dawn Kingston. 2006. "The Effects of Exercise on Body Weight and Composition in Breast Cancer Survivors: An Integrative Systematic Review." *Oncology Nursing Forum* 33 (5):937-950.

Innes, Ev. 1999. "Handgrip strength testing: A review of the literature." *Australian Occupational Therapy Journal* 46 (3):120-140. doi: 10.1046/j.1440-1630.1999.00182.x.

Irwin, M L , A McTiernan, R N Baumgartner, K B Baumgartner, L Bernstein, F D Gilliland, and R Ballard-Barbash. 2005. "Changes in body fat and weight after a breast cancer diagnosis:

influence of demographic, prognostic, and lifestyle factors." *Journal of Clinical Oncology* 23 (4):774-782.

Irwin, M L, K Varma, Marty Alvarez-Reeves, Lisa Cadmus, A Wiley, G C Chung, Loretta DiPietro, S T Mayne, and Herbert Yu. 2009. "Randomised Controlled Trial of Aerobic Exercise on Insulin and Insulin-like Growth Factors in Breast Cancer Survivors: The Yale Exercise and Survivorship Study " *Cancer Epidemiology, Biomarkers & Prevention* 18:306-313.

Irwin, M. L., D. Crumley, A. McTiernan, L. Bernstein, R. Baumgartner, F. D. Gilliland, A. Kriska, and R. Ballard-Barbash. 2003. "Physical activity levels before and after a diagnosis of breast carcinoma." *Cancer* 97 (7):1746-1757. doi: 10.1002/cncr.11227.

Irwin, Melinda L., Marty Alvarez-Reeves, Lisa Cadmus, Eileen Mierzejewski, Susan T. Mayne, Herbert Yu, Gina G. Chung, Beth Jones, M. Tish Knobf, and Loretta DiPietro. 2009. "Exercise Improves Body Fat, Lean Mass, and Bone Mass in Breast Cancer Survivors." *Obesity* 17 (8):1534-1541.

Irwin, Melinda L., Anne McTiernan, Leslie Bernstein, Frank D. Gilliland, Richard Baumgartner, Kathy Baumgartner, and Rachel Ballard-Barbash. 2004. "Physical activity levels among breast cancer survivors." *Medicine and science in sports and exercise* 36 (9):1484.

Irwin, Melinda L., Anne McTiernan, JoAnn E. Manson, Cynthia A. Thomson, Barbara Sternfeld, Marcia L. Stefanick, Jean Wactawski-Wende, Lynette Craft, Dorothy Lane, Lisa W. Martin, and Rowan Chlebowski. 2011. "Physical Activity and Survival in Postmenopausal Women with Breast Cancer: Results from the Women's Health Initiative." *Cancer Prevention Research* 4 (4):522-529. doi: 10.1158/1940-6207.capr-10-0295.

Jaburek, M, M Varecha, RE Gimeno, M Dembski, P Jezek, M Zhang, P Burn, LA Tartaglia, and KD Garlid. 1999. "Transport function and regulation of mitochondrial uncoupling proteins 2 and 3." *J Biol Chem* 274:26003 - 26007.

Jakicic, John M, and Amy D Otto. 2005. "Physical activity considerations for the treatment and prevention of obesity." *The American Journal of Clinical Nutrition* 82 (1):226S-229S.

Jen, K. L. Catherine, Zora Djuric, Nora M. DiLaura, Anne Buison, Jennifer N. Redd, Vera Maranci, and William M. Hryniuk. 2004. "Improvement of Metabolism among Obese Breast Cancer Survivors in Differing Weight Loss Regimens[ast][ast]." *Obesity* 12 (2):306-312.

Jensen, G. L. 2008. "Inflammation: roles in aging and sarcopenia." *Journal of Parenteral and Enteral Nutrition* 32 (6):656-659.

Jones, L W, N D Eves, M Haykowsky, A A Joy, and P S Douglas. 2008. "Cardiorespiratory exercise testing in clinical oncology research: systematic review and practice recommendations." *Lancet Oncology* 9:757-765.

Kabir, Morvarid, Geraldine Skurnik, Nadia Naour, Valeria Pechtner, Emmanuelle Meugnier, Sophie Rome, Annie Quignard-Boulange, Hubert Vidal, Gerard Slama, Karine Clement, Michele Guerre-Millo, and Salwa W Rizkalla. 2007. "Treatment for 2 mo with n 3 polyunsaturated fatty acids reduces adiposity and some atherogenic factors but does not improve insulin sensitivity in women with type 2 diabetes: a randomised controlled study." *Am J Clin Nutr* 86 (6):1670-1679.

Kalogeropoulos, Nick, Demosthenes B. Panagiotakos, Christos Pitsavos, Christina Chrysohoou, Georgia Rousinou, Marina Toutouza, and Christodoulos Stefanadis. 2010. "Unsaturated fatty acids are inversely associated and n-6/n-3 ratios are positively related to inflammation and coagulation markers in plasma of apparently healthy adults." *Clinica Chimica Acta* 411 (7):584-591. doi: 10.1016/j.cca.2010.01.023.

Kim, C, D-H Kang, and J-W Park. 2009. "A Meta-Analysis of Aerobic Exercise Interventions for Women With Breast Cancer." *Western Journal of Nursing Research* 31 (4):437-461.

Kopp, H. P., C. W. Kopp, A. Festa, K. Krzyzanowska, S. Kriwanek, E. Minar, R. Roka, and G. Schernthaner. 2003. "Impact of Weight Loss on Inflammatory Proteins and Their Association With the Insulin Resistance Syndrome in Morbidly Obese Patients." *Arteriosclerosis, Thrombosis, and Vascular Biology: Journal of the American Heart Association* 23 (6):1042-1047. doi: 10.1161/01.ATV.0000073313.16135.21.

Krebs, J. D., L. M. Browning, N. K. McLean, J. L. Rothwell, G. D. Mishra, C. S. Moore, and S. A. Jebb. 2006. "Additive benefits of long-chain n-3 polyunsaturated fatty acids and weight-loss in the management of cardiovascular disease risk in overweight hyperinsulinaemic women." *Int J Obes* 30 (10):1535-1544. doi: http://www.nature.com/ijo/journal/v30/n10/suppinfo/0803309s1.html.

Kroenke, C H, W Y Chen, B Rosner, and M D Holmes. 2005. "Weight, weight gain, and survival after breast cancer diagnosis." *Journal of Clinical Oncology* 23 (7):1370-1378.

Kuczmarski, R J, M T Fanelli, and G G Koch. 1987. "Ultrasonic assessment of body composition in obese adults: overcoming the limitations of the skinfold caliper." *The American Journal of Clinical Nutrition* 45 (4):717-24.

Kumar, N, K A Allen, D Riccardi, B B Bercu, A Cantor, S Minton, L Balducci, and P B Jacobsen. 2004. "Fatigue, weight gain, lethargy and amenorrhea in breast cancer patients on chemotherapy: is subclinical hypothyroidism the culprit?" *Breast Cancer Research and Treatment* 83 (2):149.

Kunesová, M , R Braunerová, P Hlavatý, E Tvrzická, B Stanková, J Skrha, J Hilgertová, M Hill, J Kopecký, M Wagenknecht, V Hainer, M Matoulek, J Parízková, A Zák, and S Svacina. 2006. "The influence of n-3 polyunsaturated fatty acids and very low calorie diet

during a short-term weight reducing regimen on weight loss and serum fatty acid composition in severely obese women." *Physiological Research* 55 (1):63-72.

Kutynec, C I, L McCargar, S I Barr, and T. Gregory Hislop. 1999. "Energy balance in women with breast cancer during adjuvant treatment." *Journal of the American Dietetic Association* 99:1222-1227.

Lahmann, Petra H., Lauren Lissner, Bo Gullberg, Håkan Olsson, and Göran Berglund. 2003. "A prospective study of adiposity and postmenopausal breast cancer risk: The Malmö diet and cancer study." *International Journal of Cancer* 103 (2):246-252. doi: 10.1002/ijc.10799.

Lee, Chong Do, Steven N Blair, and Andrew S Jackson. 1999. "Cardiorespiratory fitness, body composition, and all-cause and cardiovascular disease mortality in men." *The American Journal of Clinical Nutrition* 69 (3):373-380.

Lee, Christine G., Molly C. Carr, Susan J. Murdoch, Ellen Mitchell, Nancy F. Woods, Mark H. Wener, Wayne L. Chandler, Edward J. Boyko, and John D. Brunzell. 2009. "Adipokines, Inflammation, and Visceral Adiposity across the Menopausal Transition: A Prospective Study." *J Clin Endocrinol Metab* 94 (4):1104-1110. doi: 10.1210/jc.2008-0701.

Lemieux, Julie, Pamela J. Goodwin, Louise J. Bordeleau, Sophie Lauzier, and Valérie Théberge. 2011. "Quality-of-Life Measurement in Randomised Clinical Trials in Breast Cancer: An Updated Systematic Review (2001–2009)." *Journal of the National Cancer Institute* 103 (3):178-231.

Libby, P., Ridker, P. M., Maseri, A. (2002). "Inflammation and Atherosclerosis." Circulation **105**(9): 1135-1143.

Ligibel, J A, N Campbell, A Partridge, W Y Chen, T Salinardi, H Chen, K Adloff, A Keshaviah, and E P Winer. 2008. "Impact of a Mixed Strength and Endurance Exercise Intervention on Insulin Levels in Breast Cancer Survivors." *Journal of Clinical Oncology* 26 (6):907-912.

Ligibel, Jennifer A., and Pamela J. Goodwin. 2012. "NEW and RENEW: Building the Case for Weight Loss in Breast Cancer." *Journal of Clinical Oncology* 30 (19):2294-2296. doi: 10.1200/jco.2012.42.5496.

Lukaski, H. 2009. "Evaluation of body composition: why and how?" *Mediterranean Journal of Nutrition and Metabolism* 2 (1):1-10.

Lynch, B M, D W Dunstan, G N Healy, E Winkler, E Eakin, and O Neville. 2010. "Objectively measured physical activity and sedentary time of breast cancer survivors, and associations with adiposity: findings from NHANES (2003–2006)." *Cancer Causes and Control* 21:283-288.

MacInnis, R J, D R English, D M Gertig, J L Hopper, and G G Giles. 2004. "Body Size and Composition and Risk of Postmenopausal Breast Cancer." *Cancer Epidemiology Biomarkers & Prevention* 13 (12):2117-2125.

MacVicar, M G, M L Winningham, and J L Nickel. 1989. "Effects of aerobic interval training on cancer patients' functional capacity." *Nursing Research* 38 (6):348-353.

Madsen, Trine, Jeppe H. Christensen, Mogens Blom, and Erik B. Schmidt. 2003. "The effect of dietary n-3 fatty acids on serum concentrations of C-reactive protein: a dose-response study." *The British journal of nutrition* 89 (4):517.

Magee, Pamela J., and Ian Rowland. 2012. "Soy products in the management of breast cancer." *Current opinion in clinical nutrition and metabolic care* 15 (6):586.

Makari-Judson, G, C H Judson, and W C Mertens. 2007. "Longitudinal Patterns of Weight Gain after Breast Cancer Diagnosis: Observations beyond the First Year." *The Breast Journal* 13:258-265.

Marfell-Jones, M, T Olds, A Stewart, and L Carter. 2007. *International Standards for Anthropometric Assessment (2007)*. 2 ed. School of Biokinetics, Recreation and Sports Science, North West University, Potchefstroom, South Africa: International Society for the Advancement of Kinanthropometry.

Martin, G S. 2004. "The interviewer-administered, open-ended diet history method for assessing usual dietary intakes in clinical research: relative and criterion validation studies." PhD, University of Wollongong Thesis Collection, University of Wollongong.

Matthews, Charles, Sara Wilcox, Cara Hanby, Cheryl Der Ananian, Sue Heiney, Tebeb Gebretsadik, and Ayumi Shintani. 2007. "Evaluation of a 12-week home-based walking intervention for breast cancer survivors." *Supportive Care in Cancer* 15 (2):203-211.

McDonald, Cameron Keith, Judith D. Bauer, and Sandra Capra. 2011. "Body Composition and Breast Cancer- the Role of Lean Body Mass." *Cancer Forum* 35 (2):102-106.

McDonald, Cameron Keith, Judy D Bauer, and Sandra Capra. 2013. "Omega-3 fatty acids and changes in LBM – alone or in synergy for better muscle health? A Review." *Canadian Journal of Physiology and Pharmacology, in press*.

McNeely, Margaret L., Kristin L. Campbell, Brian H. Rowe, Terry P. Klassen, John R. Mackey, and Kerry S. Courneya. 2006. "Effects of exercise on breast cancer patients and survivors: a systematic review and meta-analysis." *Canadian Medical Association Journal* 175 (1):34-41.

Meeske, K. 2007. "Fatigue in breast cancer survivors two to five years post diagnosis: a HEAL Study Report." *Quality of Life Research* 16 (6):947-960.

Mefferd, K, J F Nichols, B Pakiz, and C L Rock. 2007. "A cognitive behavioral therapy intervention to promote weight loss improves body composition and blood lipid profiles among overweight breast cancer survivors." *Breast Cancer Research and Treatment* 104:145-152.

Mellemkjaer, Lene, Janne Bigaard, Anne Tjonneland, Jane Christensen, Birthe Thomsen, Christoffer Johansen, Kim Overvad, and Jorgen H. Olsen. 2006. "Body Composition and Breast Cancer in Postmenopausal Women: A Danish Prospective Cohort Study[ast]." *Obesity* 14 (10):1854-1862.

Micallef, M. A., I. A. Munro, and M. L. Garg. 2009. "An inverse relationship between plasma n-3 fatty acids and C-reactive protein in healthy individuals." *European Journal of Clinical Nutrition* 63 (9):1154-1156. doi: 10.1038/ejcn.2009.20.

Micallef, Michelle A., and Manohar L. Garg. 2009. "Anti-inflammatory and cardioprotective effects of n-3 polyunsaturated fatty acids and plant sterols in hyperlipidemic individuals." *Atherosclerosis* 204 (2):476-482. doi: 10.1016/j.atherosclerosis.2008.09.020.

Mills, Paul J., Sonia Ancoli-Israel, Barbara Parker, Loki Natarajan, Suzi Hong, Shamini Jain, Georgia R. Sadler, and Roland von Känel. 2008. "Predictors of inflammation in response to anthracycline-based chemotherapy for breast cancer." *Brain, Behavior, and Immunity* 22 (1):98-104. doi: DOI: 10.1016/j.bbi.2007.07.001.

Mitch, William E., and Alfred L. Goldberg. 1996. "Mechanisms of Muscle Wasting — The Role of the Ubiquitin–Proteasome Pathway." *New England Journal of Medicine* 335 (25):1897-1905. doi: doi:10.1056/NEJM199612193352507.

Montagnani, A, S Gonnelli, A Cadirni, C Caffarelli, K Del Santo, C Pieropan, M S Campagna, M Montomoli, R Petrioli, and R Nuti. 2008. "The effects on lipid serum levels of a 2-year adjuvant treatment with exemestane after tamoxifen in postmenopausal women with early breast cancer." *European Journal of Internal Medicine* 19:592-597.

Montagnani, A., R. Nuti, S. Gonnelli, A. Cadirni, C. Caffarelli, K. Del Santo, C. Pieropan, M. S. Campagna, M. Montomoli, and R. Petrioli. 2008. "The effects on lipid serum levels of a 2-year adjuvant treatment with exemestane after tamoxifen in postmenopausal women with early breast cancer." *European Journal of Internal Medicine* 19 (8):592-597. doi: 10.1016/j.ejim.2007.05.016.

Morey, Miriam C., Denise C. Snyder, Richard Sloane, Harvey Jay Cohen, Bercedis Peterson, Terryl J. Hartman, Paige Miller, Diane C. Mitchell, and Wendy Demark-Wahnefried. 2009. "Effects of Home-Based Diet and Exercise on Functional Outcomes Among Older, Overweight Long-term Cancer Survivors: RENEW: A Randomised Controlled Trial." *JAMA* 301 (18):1883-1891. doi: 10.1001/jama.2009.643.

Mourtzakis, M , and M Bedbrook. 2009. "Muscle atrophy in cancer: a role for nutrition and exercise." *Applied Physiology, Nutrition and Metabolism* 34 (5):950-956.

Muhammad, Kamran I., Thomas Morledge, Ravish Sachar, Annette Zeldin, Kathy Wolski, and Deepak L. Bhatt. 2011. "Treatment with ω-3 fatty acids reduces serum C-reactive protein concentration." *Clinical Lipidology* 6 (6):723-729. doi: 10.2217/clp.11.54.

Mulero Portela, A L , C L Santaella, C C Gómez, and A Burch. 2008. "Feasibility of an Exercise Program for Puerto Rican Women who are Breast Cancer Survivors." *Rehabilitation Oncology* 26 (2):20-31.

Munro, I E, and M L Garg. 2012. "Dietary supplementation with n-3 PUFA does not promote weight loss when combined with a very-low-energy diet." *British Journal of Nutrition* FirstView:1-9. doi: doi:10.1017/S0007114511006817.

Murphy, Rachel A., Marina Mourtzakis, Quincy S. C. Chu, Vickie E. Baracos, Tony Reiman, and Vera C. Mazurak. 2011. "Nutritional intervention with fish oil provides a benefit over standard of care for weight and skeletal muscle mass in patients with nonsmall cell lung cancer receiving chemotherapy." *Cancer* 117 (8):1775-1782. doi: 10.1002/cncr.25709.

Murphy, Rachel A., Marina Mourtzakis, and Vera C. Mazurak. 2012. "n-3 polyunsaturated fatty acids: the potential role for supplementation in cancer." *Current opinion in clinical nutrition and metabolic care* 15 (3):246. doi: 10.1097/MCO.0b013e328351c32f.

Mustian, K M , J A Katula, and H Zhao. 2006. "A pilot study to assess the influence of tai chi chuan on functional capacity among breast cancer survivors." *Journal of Supportive Oncology* 4 (3):139-145.

Mustian, Karen M., Jeffrey A. Katula, Diane L. Gill, Joseph A. Roscoe, David Lang, and Karen Murphy. 2004. "Tai Chi Chuan, health-related quality of life and self-esteem: A randomised trial with breast cancer survivors." *Supportive Care in Cancer* 12 (12):871-876.

Mutrie, Nanette, Anna M. Campbell, Fiona Whyte, Alex McConnachie, Carol Emslie, Laura Lee, Nora Kearney, Andrew Walker, and Diana Ritchie. 2007. "Benefits of supervised group exercise programme for women being treated for early stage breast cancer: pragmatic randomised controlled trial." *BMJ (Clinical research ed.)* 334 (7592):517-520. doi: 10.1136/bmj.39094.648553.AE.

Nana, A., et al. (2014). "Methodology Review: Using Dual-Energy X-ray Absorptiometry (DXA) for the Assessment of Body Composition in Athletes and Active People." *International Journal of Sport Nutrition and Exercise Metabolism* e-published ahead of print. DOI: : http://dx.doi.org/10.1123/ijsnem.2013-0228

National Breast Cancer Foundation. 2012. "Nutrition and Physical Activity http://www.nationalbreastcancer.org/breast-cancer-nutrition." Accessed 15th November.

Neuhouser, Marian L., Yvonne Schwarz, Chiachi Wang, Kara Breymeyer, Gloria Coronado, Chin-Yun Wang, Karen Noar, Xiaoling Song, and Johanna W. Lampe. 2012. "A low-glycemic load diet reduces serum C-reactive protein and modestly increases adiponectin in overweight and obese adults." *The Journal of nutrition* 142 (2):369-374. doi: 10.3945/jn.111.149807.

Newman, Anne B., Varant Kupelian, Marjolein Visser, Eleanor M. Simonsick, Bret H. Goodpaster, Stephen B. Kritchevsky, Frances A. Tylavsky, Susan M. Rubin, and Tamara B. Harris. 2006. "Strength, But Not Muscle Mass, Is Associated With Mortality in the Health, Aging and Body Composition Study Cohort." *The Journals of Gerontology Series A: Biological Sciences and Medical Sciences* 61 (1):72-77.

Nichols, H B, A Trentham-Dietz, K M Egan, L Titus-Ernstoff, M D HOlmes, A J Bersch, C N Holick, J M Hampton, M J Stampfer, W C WIllett, and P A Newcomb. 2009. "Body Mass Index Before and After Breast Cancer Diagnosis: Associations with All-Cause, Breast Cancer, and Cardiovascular Disease Mortality." *Cancer Epidemiology, Biomarkers & Prevention* 18:1403-1409. doi: 10.1158/1055-9965.EPI-08-1094

Nicole Culos-Reed, S., Linda E. Carlson, Lisa M. Daroux, and Susi Hately-Aldous. 2006. "A pilot study of yoga for breast cancer survivors: physical and psychological benefits." *Psycho-Oncology* 15 (10):891-897. doi: 10.1002/pon.1021.

Nikander, Riku, Harri Sievänen, Katriina Ojala, Tiina Oivanen, Pirkko-Liisa Kellokumpu-Lehtinen, and Tiina Saarto. 2007. "Effect of a vigorous aerobic regimen on physical performance in breast cancer patients — a randomised controlled pilot trial." *Acta Oncologica* 46 (2):181-186. doi: doi:10.1080/02841860600833145.

Niraula, Saroj, Alberto Ocana, Marguerite Ennis, and PamelaJ Goodwin. 2012. "Body size and breast cancer prognosis in relation to hormone receptor and menopausal status: a meta-analysis." *Breast Cancer Research and Treatment* 134 (2):769-781. doi: 10.1007/s10549-012-2073-x.

Niu, Kaijun, Atsushi Hozawa, Shinichi Kuriyama, Kaori Ohmori-Matsuda, Taichi Shimazu, Naoki Nakaya, Kazuki Fujita, Ichiro Tsuji, and Ryoichi Nagatomi. 2006. "Dietary long-chain n-3 fatty acids of marine origin and serum C-reactive protein concentrations are associated in a population with a diet rich in marine products." *The American journal of clinical nutrition* 84 (1):223.

Noreen, E E, M J Sass, M L Crowe, V A Pabon, J Brandauer, and L K Averill. 2010. "Effects of supplemental fish oil on resting metabolic rate, body composition, and salivary cortisol in healthy adults." *Journal of the International Society of Sports Nutrition* 8 (7):31. doi: 10.1186/1550-2783-7-31.

Norgan, N G, and J V Durnin. 1980. "The effect of 6 weeks of overfeeding on the body weight, body composition, and energy metabolism of young men." *The American Journal of Clinical Nutrition* 33 (5):978-88.

Nuri, Reza, Mehrzad Moghaddasi, Arsalan Damirchi, Hamid Emami, MohammadReza Kordi, Nader Rahnama, and Farhad Rahmani-Nia. 2012. "Effect of combination exercise training on metabolic syndrome parameters in postmenopausal women with breast cancer." *Journal of Cancer Research and Therapeutics* 8 (2):238. doi: 10.4103/0973-1482.98977.

Oh, B., P. Butow, B. Mullan, S. Clarke, P. Beale, N. Pavlakis, E. Kothe, L. Lam, and D. Rosenthal. 2010. "Impact of Medical Qigong on quality of life, fatigue, mood and inflammation in cancer patients: a randomised controlled trial." *Annals of Oncology* 21 (3):608-614. doi: 10.1093/annonc/mdp479.

Ohira, Tetsuya, Kathryn H. Schmitz, Rehana L. Ahmed, and Douglas Yee. 2006. "Effects of weight training on quality of life in recent breast cancer survivors." *Cancer* 106 (9):2076-2083. doi: 10.1002/cncr.21829.

Pakiz, Bilgé, Shirley Flatt, Wayne Bardwell, Cheryl Rock, and Paul Mills. 2011. "Effects of a Weight Loss Intervention on Body Mass, Fitness, and Inflammatory Biomarkers in Overweight or Obese Breast Cancer Survivors." *International Journal of Behavioral Medicine* 18 (4):333-341. doi: 10.1007/s12529-010-9079-8.

Pan, Sai Yi, Christine Cameron, Marie Desmeules, Howard Morrison, Cora Lynn Craig, and Xiaohong Jiang. 2009. "Individual, social, environmental, and physical environmental correlates with physical activity among Canadians: a cross-sectional study." *BMC public health* 9 (1):21-21. doi: 10.1186/1471-2458-9-21.

Pedersen, Bente K., and Mark A. Febbraio. 2012. "Muscles, exercise and obesity: skeletal muscle as a secretory organ." *Nature reviews. Endocrinology* 8 (8):457. doi: 10.1038/nrendo.2012.49.

Pierce, Brandon L., Rachel Ballard-Barbash, Leslie Bernstein, Richard N. Baumgartner, Marian L. Neuhouser, Mark H. Wener, Kathy B. Baumgartner, Frank D. Gilliland, Bess E. Sorensen, Anne McTiernan, and Cornelia M. Ulrich. 2009. "Elevated Biomarkers of Inflammation Are Associated With Reduced Survival Among Breast Cancer Patients." *J Clin Oncol* 27 (21):3437-3444. doi: 10.1200/jco.2008.18.9068.

Pierce, Brandon, Marian Neuhouser, Mark Wener, Leslie Bernstein, Richard Baumgartner, Rachel Ballard-Barbash, Frank Gilliland, Kathy Baumgartner, Bess Sorensen, Anne McTiernan, and Cornelia Ulrich. 2009. "Correlates of circulating C-reactive protein and serum amyloid A concentrations in breast cancer survivors." *Breast Cancer Research and Treatment* 114 (1):155-167.

Pierce, John P., Susan Faerber, Fred A. Wright, Cheryl L. Rock, Vicky Newman, Shirley W. Flatt, Sheila Kealey, Vicky E. Jones, Bette J. Caan, Ellen B. Gold, Mary Haan, Kathryn A. Hollenbach, Lovell Jones, James R. Marshall, Cheryl Ritenbaugh, Marcia L. Stefanick, Cynthia Thomson, Linda Wasserman, Loki Natarajan, Ronald G. Thomas, and Elizabeth A. Gilpin. 2002. "A randomised trial of the effect of a plant-based dietary pattern on additional breast cancer events and survival:: the Women's Healthy Eating and Living (WHEL) Study." *Controlled Clinical Trials* 23 (6):728-756. doi: Doi: 10.1016/s0197-2456(02)00241-6.

Pierce, John P., Loki Natarajan, Bette J. Caan, Barbara A. Parker, E. Robert Greenberg, Shirley W. Flatt, Cheryl L. Rock, Sheila Kealey, Wael K. Al-Delaimy, Wayne A. Bardwell, Robert W. Carlson, Jennifer A. Emond, Susan Faerber, Ellen B. Gold, Richard A. Hajek, Kathryn Hollenbach, Lovell A. Jones, Njeri Karanja, Lisa Madlensky, James Marshall, Vicky A. Newman, Cheryl Ritenbaugh, Cynthia A. Thomson, Linda Wasserman, and Marcia L. Stefanick. 2007. "Influence of a Diet Very High in Vegetables, Fruit, and Fiber and Low in Fat on Prognosis Following Treatment for Breast Cancer: The Women's Healthy Eating and Living (WHEL) Randomised Trial." *JAMA* 298 (3):289-298. doi: 10.1001/jama.298.3.289.

Pinto, Bernardine M., Matthew M. Clark, Nancy C. Maruyama, and Susan I. Feder. 2003. "Psychological and fitness changes associated with exercise participation among women with breast cancer." *Psycho-Oncology* 12 (2):118-126.

Pinto, Bernardine M., Georita M. Frierson, Carolyn Rabin, Joseph J. Trunzo, and Bess H. Marcus. 2005. "Home-Based Physical Activity Intervention for Breast Cancer Patients." *J Clin Oncol* 23 (15):3577-3587. doi: 10.1200/jco.2005.03.080.

Pischon, Tobias, Susan E. Hankinson, Gökhan S. Hotamisligil, Nader Rifai, Walter C. Willett, and Eric B. Rimm. 2003. "Habitual Dietary Intake of n-3 and n-6 Fatty Acids in Relation to Inflammatory Markers Among US Men and Women." *Circulation* 108 (2):155-160. doi: 10.1161/01.cir.0000079224.46084.c2.

Ploeger, H E, T Takken, M H G de Greef, and B W Timmons. 2009. "The effects of acute and chronic exercise on inflammatory markers in children and adults with a chronic inflammatory disease: a systematic review." *Exercise immunology in chronic inflammatory disease* 15:6-41.

Poudel-Tandukar, Kalpana, Akiko Nanri, Yumi Matsushita, Satoshi Sasaki, Masanori Ohta, Masao Sato, and Tetsuya Mizoue. 2009. "Dietary intakes of alpha-linolenic and linoleic acids are inversely associated with serum C-reactive protein levels among Japanese men." *Nutrition research (New York, N.Y.)* 29 (6):363.

Prado, Carla M.M., Vickie E. Baracos, Linda J. McCargar, Tony Reiman, Marina Mourtzakis, Katia Tonkin, John R. Mackey, Sheryl Koski, Edith Pituskin, and Michael B. Sawyer. 2009. "Sarcopenia as a Determinant of Chemotherapy Toxicity and Time to Tumor Progression in Metastatic Breast Cancer Patients Receiving Capecitabine Treatment." *Clinical Cancer Research* 15 (8):2920-2926. doi: 10.1158/1078-0432.ccr-08-2242.

Protani, M, M Coory, and J H Martin. 2010. "Effect of obesity on survival of women with breast cancer: systematic review and meta-analysis." *Breast Cancer Research and Treatment* 123:627-635.

Rabin, C. 2009. "Promoting Lifestyle Change Among Cancer Survivors: When Is the Teachable Moment? ." *American Journal of Lifestyle Medicine* 3:369-378.

Renehan, A G, M Tyson, M Egger, R F Heller, and M Zwahlen. 2008. "Body-mass index and incidence of cancer: a systematic review and meta-analysis of prospective observational studies." *The Lancet* 371:569-578.

Rhea, M R, B A Alvar, L N Burkett, and S D Ball. 2003. "A Meta-Analysis to Determine the Dose Response for Strength Development." *Medicine and science in sports and exercise* 35 (3):456-464.

Robien, Kim, Wendy Demark-Wahnefried, and Cheryl L. Rock. 2011. "Evidence-Based Nutrition Guidelines for Cancer Survivors: Current Guidelines, Knowledge Gaps, and Future Research Directions." *Journal of the American Dietetic Association* 111 (3):368-375. doi: DOI: 10.1016/j.jada.2010.11.014.

Robinson, M J, N A Burd, L Breen, Y Y Rerecich, A J Hector, S K Baker, and S M Phillips. 2013. "Dose-dependent responses of myofibrillar protein synthesis with beef ingestion are enhanced with resistance exercise in middle-aged men." *Applied Physiology in Nutrition and Metabolism* 38 (120-125).

Robinson, S M, K A Jameson, S F Batelaan, H J Martin, H E Syddall, E M Dennison, C Cooper, and A A Sayer. 2008. "Diet and its relationship with grip strength in community-dwelling older men and women: the Hertford- shire cohort study." *J Am Geriatr Soc* 56:84-90.

Rocha, Paulo M., José T. Barata, Pedro J. Teixeira, Robert Ross, and Luís B. Sardinha. 2008. "Independent and opposite associations of hip and waist circumference with metabolic syndrome components and with inflammatory and atherothrombotic risk factors in overweight and obese women." *Metabolism: clinical and experimental* 57 (10):1315-1322. doi: 10.1016/j.metabol.2008.01.003.

Rock, C L, and W Demark-Wahnefried. 2002. "Nutrition and survival after the diagnosis of breast cancer: A review of the evidence." *Journal of Clinical Oncology* 20 (15):3302-3316.

Rock, Cheryl L., Michael Naughton, Bilgé Pakiz, Barbara A. Parker, Rebecca L. Sedjo, Holly Wyatt, Tim E. Byers, Graham A. Colditz, Wendy Demark-Wahnefried, Patricia A. Ganz, Kathleen Y. Wolin, Anthony Elias, Helen Krontiras, Jingxia Liu, Exercise, Recovery Nutrition to Enhance, and Group Good Health for You Trial. 2013. "Reducing breast cancer recurrence with weight loss, a vanguard trial: the Exercise and Nutrition to Enhance Recovery and Good Health for You (ENERGY) Trial." *Contemporary clinical trials* 34 (2):282-295. doi: 10.1016/j.cct.2012.12.003.

Rodacki, Cintia LN, André LF Rodacki, Gleber Pereira, Katya Naliwaiko, Isabela Coelho, Daniele Pequito, and Luiz Cléudio Fernandes. 2012. "Fish-oil supplementation enhances the effects of strength training in elderly women." *The American Journal of Clinical Nutrition* 95 (2):428-436. doi: 10.3945/ajcn.111.021915.

Rogers, L Q, P Hopkins-Price, S Vicari, R Pamenter, K S Courneya, S Markwell, S Verhulst, K Hoelzer, C Naritoku, L Jones, G Dunnington, V Lanzotti, J Wynstra, L Shah, B Edson, A Graff, and M Lowy. 2009. "A Randomised Trial to Increase Physical Activity in Breast Cancer Survivors." *Medicine & Science in Sport and Exercise* 41 (4):935-946.

Rolland, Yves, Valérie Lauwers-Cances, Marco Pahor, Judith Fillaux, Hélène Grandjean, and Bruno Vellas. 2004. "Muscle strength in obese elderly women: effect of recreational physical activity in a cross-sectional study." *The American Journal of Clinical Nutrition* 79 (4):552-557.

Ronco, A L, H Boeing, E De Stefani, M Schulz, M Schulze, and T Pischon. 2009. "A case-control study on fat-to-muscle ration and risk of breast cancer." *Nutrition and Cancer* 61 (4):466-474.

Rooney, M, and A Wald. 2007. "Interventions for the Management of Weight and Body Composition Changes in Women With Breast Cancer." *Clinical Journal of Oncology Nursing* 11 (1):41-52.

Rose, D. P., D. Komninou, and G. D. Stephenson. 2004. "Obesity, adipocytokines, and insulin resistance in breast cancer." *Obesity Reviews* 5 (3):153-165. doi: 10.1111/j.1467-789X.2004.00142.x.

Ruiz, J r, X Sui, F Lobelo, James Morrow Jr, Allen Jackson, and Michael Sjöström. 2008. "Association between muscular strength and mortality in men: prospective cohort study." *British Medical Journal (Clinical Research Ed)* 337 (7661):a439-95.

Rutishauser, Ingrid H. E. 2005. "Dietary intake measurements." *Public health nutrition* 8 (7A):1100-1107. doi: 10.1079/PHN2005798.

Saarto, T., H. Sievänen, P. Kellokumpu-Lehtinen, R. Nikander, L. Vehmanen, R. Huovinen, H. Kautiainen, S. Järvenpää, H. Penttinen, M. Utriainen, A. Jääskeläinen, A. Elme, J. Ruohola,

T. Palva, H. Vertio, M. Rautalahti, M. Fogelholm, R. Luoto, and C. Blomqvist. 2011. "Effect of supervised and home exercise training on bone mineral density among breast cancer patients. A 12-month randomised controlled trial." *Osteoporosis International*:1-12. doi: 10.1007/s00198-011-1761-4.

Saquib, N. , L. Natarajan, C.L. Rock, S.W. Flatt, L. Madlensky, S. Kealey, and J.P. Pierce. 2008. "The impact of a long-term reduction in dietary energy on body weight within a randomised diet trial." *Nutrition and Cancer* 60 (1):31-38.

Schmitz, K , J Holtzman, K Courneya, L Masse, S Duval, and R Kane. 2005. "Controlled physical activity trials in cancer survivors: A systematic review and meta-analysis." *Cancer Epidemiology Biomarkers & Prevention* 14:1588-1595.

Schmitz, K H, R L Ahmed, P J Hannan, and D Yee. 2005. "Safety and Efficacy of Weight Training in Recent Breast Cancer Survivors to Alter Body Composition, Insulin, and Insulin-Like Growth Factor Axis Proteins." *Cancer Epidemiology, Biomarkers & Prevention* 14 (7):1672-1680.

Schmitz, K H, R L Ahmed, A Troxel, A Cheville, R Smith, L Lewis-Grant, C Bryan, C T Williams-Smith, and Q P Greene. 2009. "Weight Lifting in Women with Breast-Cancer-Related Lymphedema." *the New England Journal of Medicine* 361 (7):664. doi: 10.1056/NEJMoa0810118.

Schmitz, K H, K S Courneya, C E Matthews, W Demark-Wahnefried, D A Galvao, Bernardine M Pinto, M L Irwin, K Y Wolin, R J Segal, A Lucia, C M Schneider, V E von Gruenigen, and Anna L Schwartz. 2010. "American College of Sports Medicine Roundtable on Exercise Guidelines for Cancer Survivors." *Medicine & Science in Sports & Exercise July* 42 (7):1409-1426.

Scott, E., A. J. Daley, H. Doll, N. Woodroofe, R. E. Coleman, N. Mutrie, H. Crank, H. J. Powers, and J. M. Saxton. 2013. "Effects of an exercise and hypocaloric healthy eating program on biomarkers associated with long-term prognosis after early-stage breast cancer: a randomised controlled trial." *Cancer Causes & Control* 24 (1):181-191. doi: 10.1007/s10552-012-0104-x.

Segal, Roanne, William Evans, Darren Johnson, Julie Smith, Sal Colletta, Jane Gayton, Stephanie Woodard, George Wells, and Robert Reid. 2001. "Structured Exercise Improves Physical Functioning in Women With Stages I and II Breast Cancer: Results of a Randomised Controlled Trial." *J Clin Oncol* 19 (3):657-665.

Sharman, J E, and M Stowasser. 2009. "Position Statement on Exercise and Hypertension: Exercise and Sports Science Australia." *Journal of Science and Medicine in Sport* 12:252-257.

Shaw, C, P Mortimer, and P A Judd. 2007a. "A randomised controlled trial of weight reduction as a treatment for breast cancer-related lympedema." *Cancer* 110:1868-1874.

Shaw, Clare, Peter Mortimer, and Patricia A. Judd. 2007b. "Randomised controlled trial comparing a low-fat diet with a weight-reduction diet in breast cancer-related lymphedema." *Cancer* 109 (10):1949-1956. doi: 10.1002/cncr.22638.

Sheean, Patricia M., Kent Hoskins, and Melinda Stolley. 2012. "Body composition changes in females treated for breast cancer: a review of the evidence." *Breast Cancer Research and Treatment* 135 (3):663-680. doi: 10.1007/s10549-012-2200-8.

Simmons, PS, JM Miles, JE Gerich, and MW Haymond. 1984. "Increased proteolysis. An effect of increases in plasma cortisol within the physiologic range." *J Clin Invest* 73:412 - 420.

Simpson, E. R., M. S. Mahendroo, J. E. Nichols, and S. E. Bulun. 1994. "Aromatase gene expression in adipose tissue: relationship to breast cancer." *International journal of fertility and menopausal studies* 39 Suppl 2:75-83.

Singer, S J. 2004. "Some Early History of Membrane Molecular Biology " *Annual Review of Physiology* 66:1-27.

Smith, G I , P Atherton, D N Reeds, B S Mohammed, D Rankin, D J Rennie, and Bettina Mittendorfer. 2011a. "Dietary omega-3 fatty acid supplementation increases the rate of muscle protein synthesis in older adults: a randomised controlled trial." *American Journal of Clinical Nutrition* 93:402-412.

Smith, Gordon I., Philip Atherton, Dominic N. Reeds, B. Selma Mohammed, Debbie Rankin, Michael J. Rennie, and Bettina Mittendorfer. 2011b. "Omega-3 polyunsaturated fatty acids augment the muscle protein anabolic response to hyperinsulinaemia-hyperaminoacidaemia in healthy young and middle-aged men and women." *Clinical science (London, England : 1979)* 121 (6):267-278.

Smith, Helen J., Pradip Mukerji, and Michael J. Tisdale. 2005. "Attenuation of Proteasome-Induced Proteolysis in Skeletal Muscle by β-Hydroxy-β-Methylbutyrate in Cancer-Induced Muscle Loss." *Cancer Research* 65 (1):277-283.

Smith, J. Kelly, Rhesa Dykes, John E. Douglas, Guha Krishnaswamy, and Steven Berk. 1999. "Long-term Exercise and Atherogenic Activity of Blood Mononuclear Cells in Persons at Risk of Developing Ischemic Heart Disease." *JAMA: The Journal of the American Medical Association* 281 (18):1722-1727. doi: 10.1001/jama.281.18.1722.

Statistics, Australian Bureau of. 2013. "Census of Population and Housing: Socio-Economic Indexes for Areas (SEIFA), Australia, 2011." Accessed 20/11/2013. http://www.abs.gov.au/ausstats/abs@.nsf/Lookup/2033.0.55.001main+features100042011.

Storlien, L. H., R. M. Robertson, J. O. Hill, G. W. Reed, A. D. Kriketos, H. Drougas, and T. A. Sharp. 2001. "Role of weight loss and polyunsaturated fatty acids in improving metabolic fitness in moderately obese, moderately hypertensive subjects." *Journal of Hypertension* 19 (10):1745-1754.

Suni, J. H., S. I. Miilunpalo, T. M. Asikainen, R. T. Laukkanen, P. Oja, M. E. Pasanen, K. Bös, and I. M. Vuori. 1998. "Safety and feasibility of a health-related fitness test battery for adults." *Physical therapy* 78 (2):134.

Tamakoshi, K., S. Zhu, T. Yoshida, H. Toyoshima, H. Yatsuya, T. Kondo, M. Ishikawa, H. Zhang, C. Murata, R. Otsuka, T. Mabuchi, and Y. Hori. 2003. "Long-term body weight variability is associated with elevated C-reactive protein independent of current body mass index among Japanese men." *International Journal of Obesity* 27 (9):1059-1065. doi: 10.1038/sj.ijo.0802386.

Thivat, E, S Thérondel, O Lapirot, C Abrial, P Gimbergues, E Gadéa, E Planchat, F Kwiatkowski, M A Mouret-Reynier, P Chollet1, and X Durando. 2011. "Weight change during chemotherapy changes the prognosis in non metastatic breast cancer for the worse." *BMC Cancer* 10:648-656.

Thomson, C A, A T Stopeck, J W Bea, Ellen Cussler, E Nardi, G Frey, and P A Thompson. 2010. "Changes in Body Weight and Metabolic Indexes in Overweight Breast Cancer Survivors Enrolled in a Randomised Trial of Low-Fat vs. Reduced Carbohydrate Diets " *Nutrition and Cancer* 62 (9):1142-1152.

Thomson, C. A., C. L. Rock, A. R. Giuliano, T. R. Newton, H. Cui, P. M. Reid, T. L. Green, and D. S. Alberts. 2005. "Longitudinal changes in body weight and body composition among women previously treated for breast cancer consuming a high-vegetable, fruit and fiber, low-fat diet." *European Journal of Nutrition* 44 (1):18-25. doi: 10.1007/s00394-004-0487-x.

Thomson, C. A., P. A. Thompson, J Wright-Bea, E Nardi, G R Frey, and A Stopeck. 2009. "Metabolic Syndrome and Elevated C-Reactive Protein in Breast Cancer Survivors on Adjuvant Hormone Therapy." *Journal of Women's Health* 18 (12):2041-2047. doi: 10.1089/jwh.2009.1365.

Thorsdottir, I., H. Tomasson, I. Gunnarsdottir, E. Gisladottir, M. Kiely, M. D. Parra, N. M. Bandarra, G. Schaafsma, and J. A. Martinez. 2007. "Randomised trial of weight-loss-diets for young adults varying in fish and fish oil content." *Int J Obes* 31 (10):1560-1566.

Tierney, A. C., J. McMonagle, D. I. Shaw, H. L. Gulseth, O. Helal, W. H. M. Saris, J. A. Paniagua, I. Golabek-Leszczynska, C. Defoort, C. M. Williams, B. Karsltrom, B. Vessby, A. Dembinska-Kiec, J. Lopez-Miranda, E. E. Blaak, C. A. Drevon, M. J. Gibney, J. A. Lovegrove, and H. M. Roche. 2010. "Effects of dietary fat modification on insulin

sensitivity and on other risk factors of the metabolic syndrome[mdash]LIPGENE: a European randomised dietary intervention study." *Int J Obes*. doi: http://www.nature.com/ijo/journal/vaop/ncurrent/suppinfo/ijo2010209s1.html.

Travers, C., S. M. O'Neill, R. King, D. Battistutta, and S. K. Khoo. 2005. "Greene Climacteric Scale: norms in an Australian population in relation to age and menopausal status." *Climacteric : the journal of the International Menopause Society* 8 (1):56-56. doi: 10.1080/13697130400013443.

Tsai, R., Dennis, L, K., Lynch, C, F., Snetselaar, L, G., Zamba, G, K., and Scott-Conner, C. (2009). "The Risk of Developing Arm Lymphedema Among Breast Cancer Survivors: A Meta-Analysis of Treatment Factors." Annals of Surgical Oncology **16**(7): 1959-1972.

Tsitouras, P D , F Gucciardo, A D Salbe, C Heward, and S M Harman. 2008. "High omega-3 fat intake improves insulin sensitivity and reduces CRP and IL6, but does not affect other endocrine axes in healthy older adults." *Hormone and Metabolic Research* 40 (3):199-205. doi: 10.1055/s-2008-1046759.

Twiss, Janice J., Nancy L. Waltman, Kris Berg, Carol D. Ott, Gloria J. Gross, and Ada M. Lindsey. 2009. "An Exercise Intervention for Breast Cancer Survivors With Bone Loss." *Journal of Nursing Scholarship* 41 (1):20-27.

Vallance, Jeffrey K.H., Kerry S. Courneya, Ronald C. Plotnikoff, Yutaka Yasui, and John R. Mackey. 2007. "Randomised Controlled Trial of the Effects of Print Materials and Step Pedometers on Physical Activity and Quality of Life in Breast Cancer Survivors." *J Clin Oncol* 25 (17):2352-2359. doi: 10.1200/jco.2006.07.9988.

van Londen, G., S. Perera, K. Vujevich, P. Rastogi, B. Lembersky, A. Brufsky, V. Vogel, and S. Greenspan. 2011. "The impact of an aromatase inhibitor on body composition and gonadal hormone levels in women with breast cancer." *Breast Cancer Research and Treatment* 125 (2):441-446. doi: 10.1007/s10549-010-1223-2.

Vance, V., M. Mourtzakis, L. McCargar, and R. Hanning. 2011. "Weight gain in breast cancer survivors: prevalence, pattern and health consequences." *Obesity Reviews* 12 (4):282-294. doi: 10.1111/j.1467-789X.2010.00805.x.

Villarini, A., P. Pasanisi, M. Raimondi, G. Gargano, E. Bruno, D. Morelli, A. Evangelista, P. Curtosi, and F. Berrino. 2012. "Preventing weight gain during adjuvant chemotherapy for breast cancer: a dietary intervention study." *Breast Cancer Research and Treatment* 135 (2):581-589. doi: 10.1007/s10549-012-2184-4.

Vukovich, M. and B. M. Peeters (2003). "Reliability of air-displacement plethysmography in detecting body composition changes after water ingestion and after creatine supplementation." Journal of Exercise Physiology online **6**(2): 115-122.

Wada, M , C J DeLong, Y H Hong, C J Rieke, I Song, R S Sidhu, C Yuan, M Warnock, A H Schmaier, C Yokoyama, E M Smyth, S J Wilson, G A FitzGerald, R M Garavito, X Sui, J W Regan, and W L Smith. 2007. "Enzymes and receptors of prostaglandin pathways with arachidonic acid-derived versus eicosapentaenoic acid-derived substrates and products." *Journal of Biological Chemistry* 282 (2):2254-66.

Waelde, Lynn C., Larry Thompson, and Dolores Gallagher-Thompson. 2004. "A pilot study of a yoga and meditation intervention for dementia caregiver stress." *Journal of clinical psychology* 60 (6):677-687. doi: 10.1002/jclp.10259.

Ward, L S. 2009. "Is BIS ready for prime time as the gold standard measure?" *Journal of Lymphoedema* 4 (2):52-56.

Wells, J. C. K. and M. S. Fewtrell (2006). "Measuring body composition." *Archives of Disease in Childhood* **91**(7): 612-617.

William, J. Kraemer, D. Vescovi Jason, S. Volek Jeff, C. Nindl Bradley, U. Newton Robert, F. Patton John, E. Dziados Joseph, N. French Duncan, and Hakkinen Keijo. 2004. "Effects of Concurrent Resistance and Aerobic Training on Load-Bearing Performance and the Army Physical Fitness Test." *Military Medicine* 169 (12):994-994.

Winningham, M L, M G MacVicar, M Bondoc, J I Anderson, and J P Minton. 1989. "Effect of aerobic exercise on body weight and composition in patients with breast cancer on adjuvant chemotherapy." *Oncology Nursing Forum* 16 (5):683-689.

Winters-Stone, Kerri, Jessica Dobek, Lillian Nail, Jill Bennett, Michael Leo, Arpana Naik, and Anna Schwartz. 2011. "Strength training stops bone loss and builds muscle in postmenopausal breast cancer survivors: a randomised, controlled trial." *Breast Cancer Research and Treatment* 127 (2):447-456. doi: 10.1007/s10549-011-1444-z.

Woolery, Alison, Hector Myers, Beth Sternlieb, and Lonnie Zeltzer. 2004. "A yoga intervention for young adults with elevated symptoms of depression." *Alternative therapies in health and medicine* 10 (2):60.

Wyke, S. M., J. Khal, A. V. Hine, S. T. Russell, and M. J. Tisdale. 2005. "Expression of the ubiquitin-proteasome pathway and muscle loss in experimental cancer cachexia." *British Journal of Cancer* 93 (7):774-780.

Yatsuya, H., R. L. Levy, R. W. Jeffery, S. L. Langer, N. Mitchell, A. P. Flood, E. M. Welsh, M. A. Jaeb, P. S. Laqua, and M. Crowell. 2011. "Changes in C-reactive protein during weight loss and the association with changes in anthropometric variables in men and women: LIFE Study." *International journal of obesity (2005)* 35 (5):684-691. doi: 10.1038/ijo.2010.200.

Yee, L D, J L Lester, R M Cole, J R Richardson, J C Hsu, Y Li, A Lehman, M A Belury, and S K Clinton. 2010. "x-3 Fatty acid supplements in women at high risk of breast cancer have

dose-dependent effects on breast adipose tissue fatty acid composition." *American Journal of Clinical Nutrition* 91:1185-1194.

Zhang, S., A. R. Folsom, T. A. Sellers, L. H. Kushi, and J. D. Potter. 1995. "Better breast cancer survival for postmenopausal women who are less overweight and eat less fat. The Iowa women's health study." *Cancer* 76 (2):275-283. doi: 10.1002/1097-0142.

APPENDIX

Appendices Table of Contents

Appendix 1

A-1.1 Ethics and Approvals

WRI Ethical Approval

UnitingCare Health

Human Research Ethics Committee

ABN: 87 842 457 440

1st Floor Moorlands House, The Wesley Hospital
451 Coronation Drive, Auchenflower Q 4066

PO Box 499 Toowong Q 4066
Phone: 3232 7500 Facsimile: 3232 7109
Email: ethics@uchealth.com.au

27th October 2010

Please quote our reference: 1034

Mr Cameron McDonald
The University of Queensland
ST LUCIA QLD 4067

Dear Mr McDonald

RESEARCH PROPOSAL: *The Muscle Mass, Omega-3, Diet, Exercise & Lifestyle (MODEL) Study: a nutrition program for women after breast cancer treatment*

I am pleased to advise that the Uniting*Care* Health Human Research Ethics Committee has reviewed the abovenamed research proposal and at its meeting on 21st October 2010 granted ethical approval, subject to changes requested by the Committee. Thank you for your response to those requirements. I am now able to confirm approval. If your project involves inpatients or the use of hospital facilities, it will be necessary for you to obtain the approval of the Director of Medical Services before commencement.

It is a strict condition of approval that any departure from the protocol detailed in the proposal submitted for approval be reported immediately to the Committee. If there is any change to the status of the project, this should be reported also.

Approval for the project is given subject to your agreement to Uniting*Care* Health requirements for the monitoring of research, which have been based on the Australian Health Ethics Committee guidelines, a copy of which is enclosed. Please note the requirement to submit a report annually or at the completion of the project, as appropriate.

With best wishes

Yours sincerely

Douglas Killer MBBS FRACP
Executive Officer

University of Queensland – Ethical approval

THE UNIVERSITY OF QUEENSLAND
Institutional Approval Form For Experiments On Humans Including Behavioural Research

Chief Investigator:	Mr Cameron McDonald
Project Title:	The Muscle Mass, Omega-3, Diet, Exercise & Lifestyle (MODEL) Study: A Nutrition Program For Women After Breast Cancer Treatment - A Randomised Trial - 31/10/2011 - AMENDMENT
Supervisor:	A/Prof Judy Bauer, Prof Sandra Capra
Co-Investigator(s):	Dr Geoffrey Beadle, Dr Michelle Palmer
Department(s):	Human Movement Studies: Nutrition and Dietetics
Project Number:	2011000079
Granting Agency/Degree:	The Wesley Research Institute
Duration:	28th February 2014

Comments:

Name of responsible Committee:-
Medical Research Ethics Committee
This project complies with the provisions contained in the *National Statement on Ethical Conduct in Human Research* and complies with the regulations governing experimentation on humans.

Name of Ethics Committee representative:-
Associate Professor Stephan Riek
Deputy Chairperson
Medical Research Ethics Committee

Date: 2/11/11 Signature:

Appendix 2

A-2.1 Approved Recruitment Materials

Approved Clinic flyers

The Wesley Research Institute
Making a difference today

The University Of Queensland
AUSTRALIA

Do **Fish oil** and **Exercise**
help with **Body Weight & Composition** after
Treatment for Breast Cancer?

the m.o.d.e.l. study
muscle mass, omega-3, diet, exercise and lifestyle

We are seeking women who have completed treatment for breast cancer to be part of this exciting new study.

All study related medical care will be at no cost to participants

If you would like to participate or interested in knowing more about the study please contact:

Contact: Mr. Cameron McDonald

Phone: 0411380566

Email: UQbreastcancerstudy@gmail.com

the m.o.d.e.l. study
muscle mass, omega-3, diet, exercise and lifestyle

Do **Fish oil** and **Exercise**
help with **Body Weight & Composition** after
Treatment for Breast Cancer?

We are seeking women who have completed treatment for breast cancer to be part of this exciting new study.

$$$

All study related medical care will be at no cost to participants

If you would like to participate or interested in knowing more about the study please contact:

Contact: Mr. Cameron McDonald

Phone: 0411380566

Email: UQbreastcancerstudy@gmail.com

Version 3 – 5/06/12

A-2.2 Approved Radio Commercial Script

CLIENT	WESLEY RESEARCH INSTITUTE	**ACC MGR**	NICOLE
KEY	WERE0712-CTA	**WRITER**	SUPPLIED / STU
LENGTH	30 SECONDS	**STATIONS / MARKETS**	4KQ

DUE DATE	6.7.12	**START DATE**	8.7.12	**END DATE**	9.8.12

REC DATE		**STUDIO / TIME**		**TALENT**	
MX & SFX					

FVO: Have you - or someone you know - been treated for breast cancer?

The Wesley Research Institute is seeking volunteers for a study to see how omega-3, nutrition and exercise affect the body after treatment.

You may be able to participate if:
You have completed treatment for breast cancer in the last 12 months, or will complete treatment by November.

If you are interested, call **The Wesley Research Institute** on **3721 1500**
Or visit **wesleyresearch.org.au**

CLIENT APPROVAL

Contract ID	96607	**Key No.**	WERE0712-CTA	**Production Fee**	$250 +gst

By approving these scripts via email or signature below, I: 1) Approve these scripts for recording and broadcast; 2) Agree to pay the above Production Fee, which covers broadcast on radio only for up to 12 months; 3) Accept that an additional Production Fee applies for changes made after my approval; 4) Acknowledge all scripts and audio remain the property of ARN.

APPROVED _____

Appendix 3

A-3.1 Participant information and data collection tools

Telephone Initial Screening Tool

The WESLEY RESEARCH INSTITUTE
Clinical Trials Centre

Telephone Screening Tool

PROTOCOL: The MODEL Study			
TRIAL SUMMARY:			
*i.e. aim of trial, healthy, disease specific, length of involvement and **3 key points** of selection criteria*			
1. Assess changes in body composition			
2. Breast cancer survivors finishing treatment in the last 12 months			
3. Involvement for 6 months, Minimum number of visits = 9 over 6 months (maximum 12)			
PARTICIPANT'S DETAILS			**GP DETAILS**:
Name:			
Sex: ☐ Female	DOB:	Age	
Contact details:			Preferred time to be contacted:
Mobile:	Home phone:		
Email address:			**SPECIALIST:**
Postal address:			
How did you hear about the study? Advertising source?			
Screening conducted by:		Date:	Time:

SELECTION CRITERIA:		
Inclusion Criteria: If response falls within the grey area please exclude participant		
Diagnosis and Rx of Breast cancer	No ☐	Yes☐
Completed treatment successfully in last 12 months	No ☐	Yes☐
Age older than 18yrs	No ☐	Yes☐
Appropriate BMI	No ☐	Yes☐
Willing to be randomized	No ☐	Yes☐
No diagnosis of diabetes or cardiovascular disease	No ☐	Yes☐
Not pregnant	No ☐	Yes☐
	No ☐	Yes☐
Exclusion Criteria: If response falls within the grey area please exclude participant		
Did the cancer spread to any other parts of your body?	No ☐	Yes☐
Has the doctor indicated you currently have a cancer?	No ☐	Yes☐
Use of cardiac or diabetic medications	No ☐	Yes☐
Planning to have a child in next 9 months	No ☐	Yes☐
	No ☐	Yes☐
	No ☐	Yes☐
	No ☐	Yes☐

Eligibility					
Not eligible but consent for database	No ☐ Yes☐	Consent form sent ☐	N/A ☐	No ☐	Yes☐
Eligible according to this phone screen	No ☐ Yes☐	Would like to be contacted		No ☐	Yes☐
PICF sent				No ☐	Yes☐

Approved participant information and informed consent

Participant Information and Consent Form

Protocol Title: The Muscle Mass, Omega-3, Diet, Exercise & Lifestyle (MODEL) Study: a nutrition program for women after breast cancer treatment

Investigators:	Associate Professor Judy Bauer, Professor Sandra Capra, Dr. Geoffrey Beadle, Dr. Nicole McCarthy, Cameron McDonald
Site:	The Wesley Research Institute
Co-ordinating centre:	The Wesley Research Institute & University of Queensland
Funding agency:	The Wesley Research Institute

Dear Participant,

This form has 2 sections:

1. Participant Information (to share information about the study with you)
2. Consent Form (for signatures if you agree to take part).

You will be provided with a copy of the signed full Participant Information and Consent Form to keep as a record.

Introduction

You have been invited to take part in this study as you have completed treatment for breast cancer in the last 12 months. A healthy lifestyle after treatment is important for ongoing good health. The purpose of this study is to compare the effects of fish oil (omega-3) when delivered alone, or in combination with a nutrition and exercise program, and to see if this affects markers of health.

Before agreeing to participate in the study, it is important that you read and understand the information on this form. It explains all the procedures involved. It also tells you what your rights are including the right to withdraw from the study at anytime. Please ask your study doctor or other research staff to explain anything that you do not understand. Take your time, and if you wish, discuss the study with your family, doctor, friends and relatives.

How many women will be in the study and how long will it last?

There will be 30-35 women in 3 groups (90-115 altogether) taking part in the study. The study will compare results of the 3 groups over 6 months.

If you agree to participate in the study you will be allocated to one of 3 groups. Which group you go into is purely a matter of chance (random allocation) and out of the study staff's control.

If you are allocated to Group 1, you will attend a 12-week nutrition and exercise group-based program specifically developed for women who have completed treatment for breast cancer;
If you are allocated to Group 2, you will receive a specific daily dose of fish oil;
If you are allocated to Group 3 you will attend the nutrition and exercise program plus receive the daily dose of fish oil.

What will happen during the study?
General timing
This study will be conducted over 6 months. The initial 3 months will require weekly or fortnightly visits to The Wesley Research Institute. After this period you will be contacted monthly to monitor your progress and update your details as necessary.

Study Assessments
All participants will be asked to complete a number of questionnaires, physical tests and a dietary report at three time points – before you start the nutrition program and at 3 and 6 months . It is estimated that the study assessments will require two hours to complete.

Week 1 –*Location: The Wesley Research Institute and The Wesley Hospital Rehabilitation Gym*

- Questionnaires will be completed while you wait for availability of the other tests. There are 5 questionnaires in total and will take 30-40 minutes to complete. These questionnaires will gather information regarding quality of life, joint pain and soreness and levels of fatigue.
- Basic body measures: Your weight, height, waist and hip circumference will be measured using a simple set of scales and standard measuring tape and stand.
- Lymphoedema Index: will be measured using a specialised instrument, that passes a very small electric current through your body. It determines the amount of fluid in your arm. You will not feel any discomfort during the measurement
- Body composition will be measured using the BodPod. This is a special piece of equipment in which you sit for 4 minutes while the measurement is being taken. It will provide you with a very accurate reading of total body fat% and muscle mass %.

Personal Interview

- Your Diet history will be gathered through an initial questionnaire and then followed up with an interview from an Accredited Practicing Dietitian. You will be asked a series of questions relating to your regular daily food intake. Amounts and frequency of foods will be requested and reference cups, bowls and plates will be used to help you accurately identify your typical food portions. This stage will take around 20 minutes to complete.

- Cardio-respiratory fitness: If your Oncologist considers this safe and appropriate for you, you will be asked to perform what is called a sub-maximal exercise test. This involves walking on a treadmill for 6-12 minutes, the incline and speed is gradually increased until you reach a predetermined heart rate (85% of your maximum heart rate), or when you decide to end the test yourself. The speed of the treadmill will be similar to a slow walk up a hill, and will build over time. Blood pressure, heart rate and blood oxygen levels will be recorded throughout the test. The highest workload will only continue for 3 minutes (normally less) and is not expected to unduly stress your body. See additional information sheet attached for full details.
- Physical Activity: You will be asked to wear a device called an accelerometer. This device measures your pattern of movement through the day and the intensity at which you perform these activities. You will be asked to wear the device for 7 days during waking hours except for times when you are showering, bathing, swimming or performing other activities that may wet the device. The device is similar in size to a box of matches and is attached at hip height with a special belt. This will be handed in at your attendance the following week.
- A Blood sample will be taken from your arm. If you do not wish to give a blood sample you will still be considered for participation in the study. You will have around 2 teaspoons of blood taken per sample. The procedure will be carried out by a specialised technician (phlebotomist) to ensure accuracy and safety.

Week 2 –Muscular endurance: These tests indicate the strength and endurance of different groups of muscles – upper body, lower body and abdominal strength

- Push up test: Is measured by the number of push-ups you can perform consecutively without stopping. Technique will be monitored for safety. If you require a garment for lymphoedema (swelling) in your arm it is essential that this garment is worn during the test.
- Abdominal crunch test: Measured by the most crunches you can perform in one minute. A set distance is marked to maintain consistency.
- 30 second sit-stand test: Measures how many times you can move from a sitting position to a standing position from a standard chair in 30 seconds.

All movements will be demonstrated before you perform them, and you may stop the test at any time.

12 Week Nutrition and Exercise Program

If you are allocated to Group 1 or 3, you will be asked to attend the 12 week nutrition and exercise program. The commitment includes 9 sessions over 12 weeks, and information presented will be relevant to nutrition and exercise considerations for optimising health after treatment for breast cancer. Sessions will involve group discussions, nutrition and exercise presentations and active

exercise. The program will be run based on cognitive behavioural therapy which is a technique that encourages goal-setting, taking steps to achieve those goals, and addresses appropriate management of barriers to these goals.

Visiting Schedule
Below is an outline of the study and your commitments if you choose to participate.

	Groups 1 & 3	Group 2
Week 1- Day 1 testing	Initial introduction and measurements as described above for Day 1	
Week 1-2	Wear accelerometer for 7 days during waking hours.	
Week 2	Hand in Accelerometer and complete a questionnaire regarding your physical activity for the previous week.	
Week 2-13	Attend 9 weekly 60 minute sessions. Wks 2, 3, 4, 5, 6, 7, 9, 11, 13.	Attend fortnightly meetings to pick up supply of fish oil capsules and check in with research team (30 min) Attendance required: Week 2, 3 (exercise testing), 5, 7, 9, 11, 13
Week 2,3 & 13	Muscular endurance tests will be carried out in week 2, 3 & 13 during the sessions	
Week 14 Day 1 testing	Measurements as described for Day 1	
Week 14-26	-Maintain prescribed capsule intake and nutrition and exercise habits. -Receive ongoing support for progress via email and internet blog for your study group.	
Week 26 & 27	Repeat of initial assessments performed at Week 1/2 & 14/15.	

Capsule consumption
Groups 1 & 3 will complete the 12-week nutrition and exercise program together. However, those who are randomly allocated to Group 1 will receive placebo (fake) fish oil capsules, while Group 3 will receive genuine fish oil capsules. Both instructors and participants will not know who has been allocated to Group 1 or Group 3 until after the study is completed. Group 2, who will not be participating in the nutrition and exercise program & will be taking genuine fish oil capsules. All participants will be strongly discouraged from taking any additional fish oil capsules for the duration of the study.

Are there any benefits to me?
You may or may not receive any direct benefit from taking part in this study. You may receive additional information about your health and the information learnt in this study may help others in future.

What are the risks?
You may not have any problems with the fish oil doses or exercise testing and training. However, others have reported:

Fish oil: gastrointestinal upset, 'fishy' aftertaste and a longer bleeding time. Taking more than the prescribed dose of fish oil may have adverse effects in

some cases. It is strongly advised you do not take more than the dose given to you and discuss any issues with CI McDonald or your Oncologist.
Exercise testing: dizziness, light-headedness, fatigue, muscle soreness, joint ache or pain

Any adverse effects related to the study will be addressed immediately so that the issue can be resolved as quickly as possible with the help of the study doctor and research team.

Will I have to pay anything to be part of the study?

You will not be paid to take part in the study. The fish oil capsules and education program will be offered at no charge. After 6 months you will need to purchase your own fish oil capsules if you wish to continue with the dose after the study. The funding body will provide remuneration for parking fees to attend the sessions. Your study doctor is not being paid to conduct this study. Details of any financial or other relevant declarations of interest of researchers of institutions can be provided to you by your study doctor if you wish.

Will my information be kept private?

Any information obtained during this study that can identify you will remain confidential and will only be used for the purpose of the study. Your personal information will only be disclosed with your permission, except where required by law. We plan to present information and results from the study at meetings or publish it in journals. Your name, and other personal information that can easily be traced back to you, will not be included in presentations and publications. Your health records and any information obtained during the study are subject to inspection (for the purpose of verifying the procedures and data) by regulatory authorities such as the Australian Government's Therapeutic Goods Administration (TGA) and authorised representatives of the funding body, and its representatives, ethics committee, or as required by law. By signing the attached Consent Form, you authorise release of, or access to, this confidential information to the relevant study personnel and regulatory authorities as noted above.

The handling, storage, transfer and destruction of your data will comply with the *Australian Privacy Act 1988 (Cth)* and any other legislation, code or guideline which applies in the jurisdiction in which the study site is located and which related to the protection of personal information.

All your data will also be securely stored at the Wesley Research Institute and the University of Queensland and eventually archived for at least 15 years. After this time it will be disposed of appropriately.

Do I have to take part in this study?

You do not have to take part in this study if you do not wish to do so. You may stop participating in the study at any time by telling the study doctor or research staff that you do not want to continue. You do not have to give a reason. Your decision whether to take part or not to take part, or take part and then withdraw, will not affect your routine treatment or your relationship with those treating you. If you decide to stop the study, no more information will be collected about you for the study. All the information you gave us before you decided to stop the study will be used for the study.

Can I be taken out of the study?
The study doctor may at any time decide to stop the study or take you out of the study for several reasons such as:

- The required number of study participants has been met.
- You have not followed the study instructions given to you.
- It is better for your health.
- Any other reasons.

Results of the study
We aim to publish the results of this study so that other interested people may have access to the information. The information will be shared with you before it is made widely available to the public. You should feel free to ask your doctor about this. A plain English summary of the study results will be made available to you if you wish.

New information arising during the project
During this study, new information about the risks and benefits of the project may become known to the study staff. If this occurs, you will be told about this new information. This new information may mean that you can no longer participate in this research. If this occurs, the person(s) supervising the research will stop your participation. In all cases, you will be offered all available care to suit your needs and medical condition.

Further information for any problems
If you require further information or if you have any problems concerning the study you can contact the investigators:
The Study Co-ordinator: Cameron McDonald (0411380566) or
Assoc Professor Judy Bauer (3232 7918), Professor Sandra Capra (334 67703) or Dr. Geoffrey Beadle (3870 4255).
If you have any complaints about any aspects of the project, the way it is being conducted or any questions about your rights as a study participant, then you may contact:
The UnitingCare Health Human Research Ethics Committee: 07 3232 7500

Ethical Guidelines
The study will be carried out according to the National Statement on Ethical Conduct in Research Involving Humans (2007) produced by the National Health and Medical Research Council of Australia. This statement has been developed to protect the interests of people who agree to participate in human research studies. This consent form and ethical aspects of this research have been approved by the Human Ethics Committee of The Wesley Hospital.

This study adheres to the Guidelines of the ethical review process of The University of Queensland. Whilst you are free to discuss your participation in this study with project staff (Cameron McDonald - 0411380566), if you would like to speak to an officer of the University not involved in the study, you may contact the Ethics Officer on 3365 3924

A-3.2 Consent Form

Protocol Title: The Breast Cancer Muscle mass, Omega-3, Diet, Exercise & Lifestyle (MODEL) Study: a nutrition program for women after breast cancer treatment

- I have read and understand the information in this Participant Information and Consent Form
- I have read and understand the information in the Preparation for Performance of Sub-maximal exercise test
- I have been well informed about the study
- I have had the chance to ask questions and my questions have been answered
- I understand that any new information that may affect my decision to be on the study will be made available to me
- I understand that I may ask questions at any time and that I am free to withdraw from this study at any time without affecting my medical treatment or relationship with my doctors
- I understand that my participation in this study may be ended by the investigator or by the funding body for reasons that would be explained
- I agree that the study sponsors affiliates and/or related companies, authorised representatives of HREC or appropriate regulatory agencies, will be granted access to original medical records for verification of clinical trial procedures and/or data without violating my confidentiality
- I consent to my information, provided that I cannot be identified by it, being passed to other bodies working with the funding body and I understand this may include other countries
- I agree that data gathered from the results of the study may be published, provided that I cannot be identified
- I agree to follow all study procedures as outlined in this Participant Information and Consent Fro
- I understand that I do not give up any of my legal rights by signing this form
- I agree that my consent is voluntary
- A copy of this signed Consent Form will be given to me

By signing this consent form you are saying that you understand the information and that you give your consent to take part in the study.

Participant's Name: _____

Participant's Signature: _____ Date: _____

I have given a verbal explanation of this study, its procedures and risks believe that the participant understood the explanation.

Investigator's Name: _____

Investigator's Signature: _____ Date: _____

A-3.3 Participant Completed Data Collection Sheets

Contact details and Coding

FORM – 1 Participant #:
Assessment code:

The *M.O.D.E.L.* Study

For women who have completed Breast Cancer treatment

Contact details

Full name: _____

D.O.B:_____

Address:_____

_____

Preferred email address: _____

Preferred phone number: _____

Secondary phone number: _____

Emergency contact (name & relationship to you): _____

Preferred phone #: _____

GP/Specialist Details:

Change to Details (if needed):

Full Name: _____

Address: _____

_____Suburb:_____Postcode: _____

Version 1 08/03/2011

Demographical data collection sheet

FORM – 3

the m.o.d.e.l. study

muscle mass, omega-3, diet, exercise and lifestyle

Participant #:
Assessment:

Please answer ALL of the following questions. Please ask one of the research team if you would like further clarification for any of the items below.

1. **What is your age in years?**

2. **What is your current marital status?** (Please circle)

   Single, Never married Defacto Married Separated Divorced Widowed

3. **Do you have any children?** (Please circle)

   YES-go to Q4 **NO** –go to Q5

4. **How many children do you have?**

5. **What is highest level of education you have completed? (**Please circle)
   - Less than high school
   - High school
   - Some University
   - University *(please specify level of achievement)*
     Bachelor Masters Doctorate
   - Some TAFE or post-schooling education
   - TAFE course
   - Graduate Certificate/Diploma
   - Other (please specify)…….

6. **What best describes your current employment status?**

   Full time Part time Casual Contractor Unemployed

7. **For how many hours on average do you work each week?**

FORM – 3

Participant #:
Assessment:

8. What is your race**?** (Please circle)
   Asian (specify region)……
   Aboriginal or Torres Strait Islander
   Caucasian
   African-American
   Asian-Pacific Islander
   Hispanic

9. Please indicate your total household annual income?

Less than $10,000	$40,000-$49,999	$80,000-$89,999
$10,000 - $19,999	$50,000-$59,999	$90,000-$99,999
$20,000-29,999	$60,000-$69,999	$100,000-$149,999
$30,000-$39,999	$70,000-$79,999	+$150,000

10. Please indicate if there is any history of the following conditions in your first degree relatives (father, mother, siblings). Tick the box that applies to you.

Condition	No history	Father	Mother	Sibling
Heart disease, heart attack, heart failure, heart surgery				
Diabetes (Type 2)				
High blood pressure				
High cholesterol				
Breast cancer				
Other cancer (describe):				

11. Current smoking habits
    a. Current smoker – How many cigarettes per day?……
    b. Past smoker – How long since your last cigarette?……
    c. Never smoked

12. Current alcohol usage
    a. Some alcohol consumed each week
    b. No alcohol consumed

Medical information collection sheet

FORM- 4

Participant #:
Assessment code:

Please fill in your answer in the far right hand box for each question.

		Answer Here
1. Date of Diagnosis (approximate)	(mm/yy)	
2. Date of surgery	Add all dates if you have had >1 surgery (mm/yy)	
3. Description of tumour Stage of cancer	**PLEASE PROVIDE DIAGNOSTIC INFO FROM YOUR ONCOLOGIST**	Stage 0, Stage I Stage IIA; Stage IIB, Stage IIIA Size of tumour (mm):
4. When did you complete surgery, chemo and/or radiotherapy	Please be specific with date (dd/mm/yy)	
5. Type of breast cancer	Please circle one or more of the options	Oestrogen Positive Oestrogen Negative HER2 +ve
6. Type of Surgery	If yes, please circle the type (s) of surgery you have had to date.	Mastectomy Double Mastectomy Breast Reconstruction Breast Conservation
7. Did you have lymph nodes removed?	Please indicate, 'YES' or 'NO' & the total number of lymph nodes removed	Yes – nodes were removed .....nodes were removed No – nodes weren't removed
8. Did you have Chemotherapy ?	Please indicate 'Yes or No' & the type of chemotherapy agent(s) used	YES NO Type..... .....
9. Did you have Taxane chemotherapy	E.g. Taxol, Paclitaxel, Onxal	YES NO
10. Menstrual cycle	Date of your last menstrual cycle: Date:_____ And circle one of the options that best relates to your menstrual cycle at this time.	Still having regular menstrual cycles Having intermittent menstrual cycles – less than 6 months apart Last menstrual cycle was 6-12 months ago Last menstrual cycle was more than 12 months ago
11. Had you experienced menopause before treatment for breast cancer?	Please answer YES or NO	Yes No
12. Did you experience menopause during treatment?	Did you have your last menstrual cycle during chemotherapy?	Yes No
13. Are you currently on hormonal therapy	Please circle one	NONE FEMARA ARIMIDEX TAMOXIFEN OTHER.....

Body composition and Physical Function Collection Sheet

FORM-8
Code #:

Time of assessment:

	Height (m)	Weight (kg)	FFM (kg)	FFM %	BFM (kg)	BFM %	Waist (cm) Narrow/ Midpoint	Hip (cm)
Measure 1								
Measure 2								
Measure 3								

L-DEX

Trial	1	2	3	Average
Affected arm (R/L)	**L-DEX – Normal/ Not**	**R: Affected Xc: Affected**	**R: Unaffected**	**Xc: Unaffected**

Feelings of Energy out of 10:

Grip Strength	**Trial 1**	**Trial 2**	**Trial 3**	**Average**
R/L Dominant Dominant arm				
Non-Dominant arm				

Measure	**RHR**	**Blood pressure sitting**	**Blood pressure standing**

Treadmill test	**Age**	**Max HR**	**85% Max**

Stage & start time	**Speed**	**Grade (%)**	**RPE** (last 5s each min)	**Heart Rate** (last 5s each min)	**Signs and Symptoms**	**Final time**
1 (0min)	**2.7**	**0**				
2 (3min)	**2.7**	**5**				
3(6min)	**2.7**	**10**				
4 (9min)	**4**	**12**				
5(12min)	**5.4**	**14**				
6(15min)	**6.7**	**16**				

Assessment 2

Push-up test		**Comments**
Sit-to-stand		

FORM-8
Code #:

Time of assessment:

Follow up assessments

Caffeine consumption today: **YES** **NO**

Feeling (energy) out of 10: **1 2 3 4 5 6 7 8 9 10**

Date of last illness: **/ / or approx:**

Food and drink log

Meal	**Food**	**Amount** (weight, cup, volume)	**Fluid intake** (including milk on cereal, water, tea, NO COFFEE/TEA)
Breakfast			
Morning tea			

QOL Questionnaires – FACT-B+4 & FACT-F subscale

FORM - 5 **FUNCTIONAL ASSESSMENT of CANCER THERAPY** Participant:
Ax Code:

Please circle or mark one number per line to indicate your response as it applies to the <u>past 7 days</u>.

	ADDITIONAL CONCERNS	**Not at all**	**A little bit**	**Some-what**	**Quite a bit**	**Very much**
B1	I have been short of breath	0	1	2	3	4
B2	I am self-conscious about the way I dress	0	1	2	3	4
B3	One or both of my arms are swollen or tender	0	1	2	3	4
B4	I feel sexually attractive	0	1	2	3	4
B5	I am bothered by hair loss	0	1	2	3	4
B6	I worry that other members of my family might someday get the same illness I have	0	1	2	3	4
B7	I worry about the effect of stress on my illness	0	1	2	3	4
B8	I am bothered by a change in weight	0	1	2	3	4
B9	I am able to feel like a woman	0	1	2	3	4
P2	I have certain parts of my body where I experience pain	0	1	2	3	4
Q6	On which side was your breast operation? Left Right (please circle one)					
B10	Movement of my arm on this side is painful	0	1	2	3	4
B11	I have a poor range of arm movements on this side	0	1	2	3	4
B12	My arm on this side feels numb	0	1	2	3	4
B13	I have stiffness of my arm on this side	0	1	2	3	4

FUNCTIONAL ASSESSMENT of CANCER THERAPY Participant:
Ax Code:

Please circle or mark one number per line to indicate your response as it applies to the <u>past 7 days</u>.

	EMOTIONAL WELL-BEING	**Not at all**	**A little bit**	**Some-what**	**Quite a bit**	**Very much**
GE1	I feel sad	0	1	2	3	4
GE2	I am satisfied with how I am coping with my illness	0	1	2	3	4
GE3	I am losing hope in the fight against my illness	0	1	2	3	4
GE4	I feel nervous	0	1	2	3	4
GE5	I worry about dying	0	1	2	3	4
GE6	I worry that my condition will get worse	0	1	2	3	4

	FUNCTIONAL WELL-BEING	**Not at all**	**A little bit**	**Some-what**	**Quite a bit**	**Very much**
GF1	I am able to work (include work at home)	0	1	2	3	4
GF2	My work (include work at home) is fulfilling	0	1	2	3	4
GF3	I am able to enjoy life	0	1	2	3	4
GF4	I have accepted my illness	0	1	2	3	4
GF5	I am sleeping well	0	1	2	3	4
GF6	I am enjoying the things I usually do for fun	0	1	2	3	4
GF7	I am content with the quality of my life right now	0	1	2	3	4

FORM - 5 **FUNCTIONAL ASSESSMENT of CANCER THERAPY** Participant:
Ax Code:

Below is a list of statements that other people with your illness have said are important. **Please circle or mark one number per line to indicate your response as it applies to the past 7 days.**

	PHYSICAL WELL-BEING	**Not at all**	**A little bit**	**Some-what**	**Quite a bit**	**Very much**
GP1	I have a lack of energy	0	1	2	3	4
GP2	I have nausea	0	1	2	3	4
GP3	Because of my physical condition, I have trouble meeting the needs of my family	0	1	2	3	4
GP4	I have pain	0	1	2	3	4
GP5	I am bothered by side effects of treatment	0	1	2	3	4
GP6	I feel ill	0	1	2	3	4
GP7	I am forced to spend time in bed	0	1	2	3	4

	SOCIAL/FAMILY WELL-BEING	**Not at all**	**A little bit**	**Some-what**	**Quite a bit**	**Very much**
GS1	I feel close to my friends	0	1	2	3	4
GS2	I get emotional support from my family	0	1	2	3	4
GS3	I get support from my friends	0	1	2	3	4
GS4	My family has accepted my illness	0	1	2	3	4
GS5	I am satisfied with family communication about my illness	0	1	2	3	4
GS6	I feel close to my partner (or the person who is my main support)	0	1	2	3	4
Q1	*Regardless of your current level of sexual activity, please answer the following question. If you prefer not to answer it, please mark this box* ☐ *and go to the next section.*					
GS7	I am satisfied with my sex life	0	1	2	3	4

FORM - 5 **FUNCTIONAL ASSESSMENT of CANCER THERAPY** Participant:
Ax Code:

Please circle or mark one number per line to indicate your response as it applies to the past 7 days.

	ADDITIONAL CONCERNS	**Not at all**	**A little bit**	**Some-what**	**Quite a bit**	**Very much**
HI7	I feel fatigued	0	1	2	3	4
HI12	I feel weak all over	0	1	2	3	4
An1	I feel listless ("washed out")	0	1	2	3	4
An2	I feel tired	0	1	2	3	4
An3	I have trouble starting things because I am tired	0	1	2	3	4
An4	I have trouble finishing things because I am tired	0	1	2	3	4
An5	I have energy	0	1	2	3	4
An7	I am able to do my usual activities	0	1	2	3	4
An8	I need to sleep during the day	0	1	2	3	4
An12	I am too tired to eat	0	1	2	3	4
An14	I need help doing my usual activities	0	1	2	3	4
An15	I am frustrated by being too tired to do the things I want to do	0	1	2	3	4
An16	I have to limit my social activity because I am tired	0	1	2	3	4

----------End of Questionnaire----------

HEALTH ASSESSMENT QUESTIONNAIRE (HAQ-DI)©

Name: _____ Date: _____

Please place an "x" in the box which best describes your abilities OVER THE PAST WEEK:

	WITHOUT ANY DIFFICULTY	WITH SOME DIFFICULTY	WITH MUCH DIFFICULTY	UNABLE TO DO
DRESSING & GROOMING				
Are you able to:				
Dress yourself, including shoelaces and buttons?	☐	☐	☐	☐
Shampoo your hair?	☐	☐	☐	☐
ARISING				
Are you able to:				
Stand up from a straight chair?	☐	☐	☐	☐
Get in and out of bed?	☐	☐	☐	☐
EATING				
Are you able to:				
Cut your own meat?	☐	☐	☐	☐
Lift a full cup or glass to your mouth?	☐	☐	☐	☐
Open a new milk carton?	☐	☐	☐	☐
WALKING				
Are you able to:				
Walk outdoors on flat ground?	☐	☐	☐	☐
Climb up five steps?	☐	☐	☐	☐

Please check any AIDS OR DEVICES that you usually use for any of the above activities:

☐ Devices used for Dressing (button hook, zipper pull, etc.)
☐ Built up or special utensils
☐ Crutches
☐ Cane
☐ Wheelchair
☐ Special or built up chair
☐ Walker

Please check any categories for which you usually need HELP FROM ANOTHER PERSON:

☐ Dressing and grooming ☐ Arising ☐ Eating ☐ Walking

Please place an "x" in the box which best describes your abilities OVER THE PAST WEEK:

	WITHOUT ANY DIFFICULTY	WITH SOME DIFFICULTY	WITH MUCH DIFFICULTY	UNABLE TO DO
HYGIENE				
Are you able to:				
Wash and dry your body?	☐	☐	☐	☐
Take a tub bath?	☐	☐	☐	☐
Get on and off the toilet?	☐	☐	☐	☐
REACH				
Are you able to:				
Reach and get down a 5 pound object (such as a bag of sugar) from above your head?	☐	☐	☐	☐
Bend down to pick up clothing from the floor?	☐	☐	☐	☐
GRIP				
Are you able to:				
Open car doors?	☐	☐	☐	☐
Open previously opened jars?	☐	☐	☐	☐
Turn faucets on and off?	☐	☐	☐	☐
ACTIVITIES				
Are you able to:				
Run errands and shop?	☐	☐	☐	☐
Get in and out of a car?	☐	☐	☐	☐
Do chores such as vacuuming or yard work?	☐	☐	☐	☐

Please check any AIDS OR DEVICES that you usually use for any of the above activities:

☐ Raised toilet seat
☐ Bathtub bar
☐ Long-handled appliances for reach
☐ Bathtub seat
☐ Long-handled appliances in bathroom
☐ Jar opener (for jars previously opened)

Please check any categories for which you usually need HELP FROM ANOTHER PERSON:

☐ Hygiene ☐ Reach ☐ Gripping and opening things ☐ Errands and chores

Your ACTIVITIES: To what extent are you able to carry out your everyday physical activities such as walking, climbing stairs, carrying groceries, or moving a chair?

COMPLETELY	MOSTLY	MODERATELY	A LITTLE	NOT AT ALL
☐	☐	☐	☐	☐

Your PAIN: How much pain have you had IN THE PAST WEEK?
On a scale of 0 to 100 (where zero represents "no pain" and 100 represents "severe pain"), please record the number below.

☐☐☐

Your HEALTH: Please rate how well you are doing on a scale of 0 to 100 (0 represents "very well" and 100 represents "very poor" health), please record the number below.

☐☐☐

Greene Climacteric Scale Questionnaire

The *M.O.D.E.L.* Study

The Greene Climacteric Scale

FORM - 10

Participant #:
Ax Code #:

Please indicate the extent to which you are bothered at the moment by any of these symptoms by placing a tick in the appropriate box.

***(Tick one box** for each item)*	**Not at all**	**A little**	**Quite a bit**	**Extremely**
a Heart beating quickly or strongly				
b Feeling tense or nervous				
c Difficulty in sleeping				
d Excitable				
e Attacks of panic				
f Difficulty in concentrating				
g Feeling tired or lacking in energy				
h Loss of interest in most things				
i Feeling unhappy or depressed				
j Crying spells				
k Irritability				
l Feeling dizzy or faint				
m Pressure or tightness in head or body				
n Parts of body feel numb or tingling				
o Headaches				
p Muscle and joint pains				
q Loss of feeling hands or feet				
r Breathing difficulties				
s Hot flushes				
t Sweating at night				
u Loss of interest in sex				

Green, J. G., *Constructing a standard climacteric scale*. Mauritas, 1998. 29:p. 25-31.

The *M.O.D.E.L.* Study

FORM - 13

The Active Australia Survey

Initials:
Code #:

Physical Activity Questionnaire

These questions are designed to determine how much physical activity you have performed in the last week.

- Answer all questions in relation to the last week (7 days)

The Active Australia Survey
The next questions are about any physical activities that you may have done in the last week:

1. In the last week, how many times have you walked continuously, for at least 10 minutes, for recreation, exercise or to get to or from places?

☐☐ Times

2. What do you estimate was the total time that you spent walking in this way in the last week?

In hours and/or minutes

☐☐☐ minutes

☐☐ hours

3. In the last week, how many times did you do any vigorous gardening or heavy work around the yard, which made you breathe harder or puff and pant?

☐☐ times

4. What do you estimate was the total time that you spent doing vigorous gardening or heavy work around the yard in the last week?

In hours and/or minutes

☐☐☐ minutes

☐☐ hours

The next questions exclude household chores, gardening or yardwork:

5. In the last week, how many times did you do any vigorous physical activity which made you breathe harder or puff and pant? (e.g. jogging, cycling, aerobics, competitive tennis)

☐☐ times

6. What do you estimate was the total time that you spent doing this vigorous physical activity in the last week?

In hours and/or minutes

☐☐☐ minutes

☐☐ hours

7. In the last week, how many times did you do any other more moderate physical activities that you have not already mentioned? (e.g. gentle swimming, social tennis, golf)

☐☐ times

8. What do you estimate was the total time that you spent doing these activities in the last week?

In hours and/or minutes

☐☐☐ minutes

☐☐ hours

To what extent do you agree or disagree with the following statements about physical activity and health?

9(a) Taking the stairs at work or generally being more active for at least 30 minutes each day is enough to improve your health.

strongly disagree	disagree	neither agree nor disagree	agree	strongly agree

9(b) Half an hour of brisk walking on most days is enough to improve your health.

strongly disagree	disagree	neither agree nor disagree	agree	strongly agree

9(c) To improve your health it is essential for you to do vigorous exercise for at least 20 minutes each time, three times a week.

strongly disagree	disagree	neither agree nor disagree	agree	strongly agree

9(d) Exercise doesn't have to be done all at one time—blocks of 10 minutes are okay.

strongly disagree	disagree	neither agree nor disagree	agree	strongly agree

9(e) Moderate exercise that increases your heart rate slightly can improve your health.

strongly disagree	disagree	neither agree nor disagree	agree	strongly agree

Participant Data Collection Package – Day 2 to 7

Home data collection instructions

the m.o.d.e.l. study
muscle mass, omega-3, diet, exercise and lifestyle

Thank you for being part of this study,

Congratulations on completing the first day of your baseline measures for the MODEL study.

Over the next week there are two very important things you need to do before coming back for the final day of your baseline measures.

1. Wear your accelerometer as much as possible during the waking hours of the day – as described in your accelerometer journal.

2. Plan to have a fasting blood sample taken at your nearest Healthscope Pathology branch within the next 7 days

3. Please complete the Diet History Questionnaire in one sitting. Fill the form out whilst thinking about your food intake over the last month

Your accelerometer package

Inside your initial package, you will find the accelerometer journal that includes instructions and 2 tables to fill out through the week.

Table 1 is used to record when you wear your accelerometer; Table 2 is for reporting the amount of time spent performing activities labelled in the boxes.

Taking your blood sample

Take the Healthscope form provided in this package to the closest Sullivan & Nicolaides Pathology lab.
The test will involve collection 10-20ml of blood that is similar to a routine blood test.
The addresses of the laboratories are listed on the sheet attached
Please ensure that for the test you:

- Have not eaten prior to the blood being taken
- Take a snack to consume after the blood test
- Do not perform any vigorous exercise the morning of the test
- Are not planning any heavy lifting for the next 24 hours

Next week...

You will be performing some muscular endurance tests and we will go through your diet history form - that will take around 30 minutes. You will also hand in your accelerometer and be given your first month's batch of capsules to start consuming.

See you then,

Cam McDonald

0411380566

UQbreastcancerstudy@gmail.com

Healthscope Pathology Blood Analysis Request Form

Healthscope
FUNCTIONAL PATHOLOGY
1868 Dandenong Rd, Clayton Vic 3168 Ph: 1300 55 44 80

Non-Medicare

Pathology Testing Request Form

Testing to be performed by Healthscope Functional Pathology only

Please note: this form must be returned with specimens for testing to proceed

Patient Details	Practitioner Details
Surname: **Given Names:**	**Name:** Mr Cameron McDonald
Address:	**Address:** University of Queensland School of Human Movement – Level 5 St Lucia Qld 4072
Postcode:	
Phone: **Mobile:**	**Phone:** 0411 380 566 **Email:** cam.mcdonald1@gmail.com
Date of Birth ...../...../..... **Sex M / F**	**Signature:**
Clinical Notes:	

Tests Requested:

1. **Red Cell Essential Fatty Acids – refer notes below regarding specimen processing**
2. **Highly Sensitive CRP**

Instructions for patients

For tests that require a blood specimen:

You will need to attend a Healthscope Pathology collection centre.

Visit www.functionalpathology.com.au or call Healthscope Functional Pathology's friendly Customer Service staff on 1300 55 44 80 to find your nearest one.

Instructions for Healthscope Pathology staff

Collection centre staff

Collection date/time	ACC branch	Staff member

Please perform collections for the **blood tests** indicated above. Samples need to be sent to Eight Mile Plains Laboratory, Qld.

Lab please prepare the Red Cell Essential Fatty Acid samples as follows and send frozen to Healthscope Functional Pathology, Clayton Laboratory, Victoria.

1. Centrifuge the blood at 3000rpm for 10 minutes
2. Remove plasma and wash red cells be resuspending in 0.9% saline, centrifuging at 3000 rpm for 10 minutes. Repeat washing process three times.
3. Store red cell fraction at -20^{O}C, send frozen samples asap to Healthscope Clayton

NO PAYMENT IS REQUIRED – all charges will be billed to the practitioner

PAYMENT: BILLING IS TO BE SENT TO THE PRACTITIONER

Optimal science | Optimal service | Optimal health

1300 55 44 80 | csfp@healthscope.com.au | www.functionalpathology.com.au

Accelerometer Manual and Wear time journal

Instructions for Accelerometer Diary

What your accelerometer is measuring.

The accelerometer is designed to measure how much, and how intensely you move during the day. This information will give you an indication of how much energy you expend.

Please wear the accelerometer as much as possible during waking hours.

Do not wear it swimming, in the shower or where it may get wet!

How to wear your accelerometer
Using the strap provided, wear the accelerometer at the front of your right hip. Make sure the strap is tight enough to not fall, but not so tight that it is uncomfortable

Below are further explanations for completing the accelerometer journal
Wake Up Time: The time you get out of bed in the morning
Accelerometer Attached: The time at which you attach the accelerometer to your waist
Accelerometer removed: Time at which the accelerometer is removed
Multiple attach and remove: If you take the accelerometer off through the day please indicate the time on and time off.
Time in bed: Time you get into bed for the night

the m.o.d.e.l. study

Accelerometer Instructions and Journal

Cameron McDonald
P: 0411380566
E: UQbreastcancerstudy@gmail.com

How to wear your accelerometer

1. Aim for 10 hours per day as a minimum
2. It's ok if you miss a day!
3. Make sure it is comfortable – options for placement: 1. Strapped on top of your belt (in line with the middle of your thigh); 2. At your waistline in the middle of your back (if option 1 is uncomfortable)
   i) The most important thing is to keep the position consistent.
4. You can wear it under or on top of your clothes
5. Try to wear it on at least one weekend day
6. If you are going out at night and don't want to wear it, thats ok. Aim to wear it for 10 hours before that time!
7. You don't have to wear it to bed. But keep it on the dresser!

Table 1 Accelerometer usage

Day No.	1	2	3	4	5	6	7
Actual Day *E.g. Monday*							
Hours of sleep (previous night)							
Wake Up Time							
Accelerometer attached							
Accelerometer removed							
Accelerometer attached							
Accelerometer removed							
Accelerometer attached							
Accelerometer removed							
Time in bed							

How much exercise are you performing – use this chart to record your organised exercise each day

Day No.	1	2	3	4	5	6	7
Vigorous exercise (mins) e.g. *Brisk walk – 25 mins + heavy gardening- 60 mins*							
Moderate exercise (mins) e.g. slow walk – 20mins							

Vigorous exercise: Physical activity that includes brisk walking, jogging, swimming, weight training, hard cycling, heavy gardening or housework that makes you breath harder and puff.

Moderate exercise: More gentle activity that is not as intense as vigorous exercise, some examples but not limited to: E.g. slow walking, social tennis, gentle cycle or swimming, golf.

Participant #: Ax Code:

The MODEL Study

Diet History Questionnaire

Created by: Smart Foods Centre University of Wollongong

Please complete the following pages while thinking about your average week of food over the last month.

Try to complete the entire questionnaire in one or two sittings. Don't spread entry out over the week.

Work through the booklet one meal at a time, and anything that happens once a month or more frequent than that should be included.

Current Medications:_____

Supplements: _____

Office use:
Interviewer: _____ Interview number: _____ Date: _____

Part 1: Breakfast
How often do you eat breakfast? _____

Breakfast Cereals/Porridge

Prompts	Type	Frequency
Corn Wheat Rice Muesli Oats		

Milk Type: _____ Serving size: _____ mls/cups Frequency: _____
Sugar/Sweetener: _____ Serving size: _____ tsp Frequency: _____

Bread/Toast/Muffins including toppings

Prompts	Type	Serving size	Frequency
White bread Wholemeal bread Rye bread Soy & Linseed bread Raisin bread Muffins Crumpets			

Prompts	Type	Serving size	Frequency
White bread Wholemeal bread Croissants			

Hot/Cooked Dishes

Prompts	Type	Serving size	Frequency
Scrambled eggs Fried eggs Poached eggs Boiled eggs Bacon Baked beans Pancakes Sausages Hash browns ***** SALT *****			

Type of oil/fat used in cooking: _____ Serving size: _____

Tea, coffee and other drinks (hot chocolate, fruit juice, smoothie)

Type:_____ Frequency:_____

_____Frequency:_____

Other Foods

Prompts	**Type**	**Serving size**	**Frequency**
Yoghurt Fruit salad Cereal Bars Protein shakes Protein powder			

Part 2: Morning Tea

Prompts	Type	Serving size	Frequency
Tea Coffee Juice Flavoured milk Yoghurt Fruit Biscuits Cake Cereal bars Muffins Chocolate			

Prompts	Type	Serving size	Frequency
Tea Coffee Juice Flavoured milk Yoghurt Fruit			

Part 3: Lunch

Sandwiches/Rolls

Prompts	Type of Bread/Roll	Serving size	Frequency
White roll/bread Wholemeal roll/bread Multigrain bread Rye bread Lebanese bread Pita bread Turkish bread			

Fillings/Toppings

Prompts	Type	Serving size	Frequency
Meat/ham/chicken Burger meat Cheese Vegemite Jam Honey Salad Mayonnaise			

Salads

Prompts	Type	Serving size	Frequency
Mixed green Potato salad Coleslaw Greek salad **Caesar salad** Bean salad **Tabouleh** Pasta salad	******* SALT ********		

Soups

Prompts	Type	Serving size	Frequency
Minestrone soup Pea & ham soup Potato & leek soup Chicken soup Cuppa soup Vegetable soup Pumpkin soup			

Hot Meals (home prepared)

Prompts	Type	Serving size	Frequency
Fish Chicken Meat Spaghetti Bolognaise Pasta <u>Takeaway foods</u> Pies Pizza Hamburgers **Hot chips**	******* SALT ********		

Prompts	Type	Serving size	Frequency
Fish Chicken			

Tea, coffee, juice, soft drink, cordial etc

Type: _____ Frequency:_____

Type:_____ Frequency:_____

Other Foods

Type	Serving size	Frequency

Part 4: Afternoon Tea

Prompts	Type	Serving Size	Frequency
Tea Coffee Juice Flavoured milk Yoghurt Fruit Biscuits Cake Cereal bars Muffins Chocolate			

Prompts	Type	Serving Size	Frequency
Tea Coffee Juice Flavoured milk Yoghurt Fruit			

Part 5: Dinner

Prompts	Type , serving size, cooking method	Frequency
Meat (steak) Chicken Fish Schnitzel Pasta Spaghetti Bolognaise Lasagne Stir fries Casseroles Stews Soups Risotto Quiche Accompaniments Potato Vegetables Accompaniments Potato Vegetables Mash Wedges Rice Cous cous Salads		

Prompts	Type , serving size, cooking method	Frequency
Meat (steak) Chicken		

Tea, coffee, juice, soft drink, cordial etc

Type:_____ Frequency:_____

Type:_____ Frequency:_____

Type:_____ Frequency:_____

Desserts

Prompts	Type	Serving size	Frequency
Ice cream Fruit Apple pie Crumbles Cake Pudding Lamingtons Cookies Biscuits			

Part 6: Evening Snack Foods

Prompts	Type	Serving size	Frequency
Tea Coffee Juice Flavoured milk Yoghurt Fruit Biscuits Cake Cereal bars Muffins Chocolate			

Part 7: Takeaway/Restaurant Meals

Prompts	Type	Serving size	Frequency
<u>McDonald's</u> quarter pounder burger fries shake Kentucky Fried Chicken fried chicken roast chicken nuggets fries Pizza pan thin-based toppings soft drink Asian food Chinese Japanese Thai Vietnamese Fish and chips battered and fried grilled potato scallops fries Other			

Prompts	Type	Serving size	Frequency
<u>McDonald's</u> quarter pounder soft drink Indian food Italian food Mexican food			

Part 8: Food Frequency Checklist (only tick required if accounted for)

Type of food	**Serving size**	**Frequency**
Milk/Flavoured Milk		
Fruit		
Fruit juice		
Softdrinks/cordials		
Alcohol		
Yoghurt		
Cheese		
Ice creams		
Crispbreads/crackers		
Biscuits		
Cakes/Scones		
Chocolate		
Chips		
Lollies		

Omega-rich foods

Type of food	**Serving size**	**Frequency**
Walnuts		
Pecans		
Mixed Nuts		
Omega eggs		
Gold'n Canola margarine		
Salmon		
Tuna		
Canned tuna/salmon		
Mackerel		
White fish varieties		
Oysters		
Other fish		
Linseed or flaxseed oil		
Red clover		
Seeds		

Soy-rich foods

Soy and Linseed Bread and Muffins		
Soy milk		
Soy beans		
Flavoured soy drinks		
Soy enriched cereals		
Tofu		
Tempeh		
Soy sauce		
Soy snacks		
Soy yoghurt		
Soy meat products		
Soy cheese		
Soy ice cream		

Part 9: Food Preparation Practices

6.1 Butter/Margarine _____ _____

What type do you usually use?

Butter
Dairy Blend
Margarine - polyunsaturated, regular
e) Margarine - monounsaturated, regular
f) Margarine - monounsaturated, reduced fat
g) Margarine - polyunsaturated, reduced fat
h) Canola margarine
i) Gold'n Canola
j) Soy margarine

6.2 Oil/Fat in Cooking_____ _____

What type of oil/fat do you use in cooking?

Butter
Dairy blend
Margarine - polyunsaturated, regular
Margarine - polyunsaturated, reduced fat
Margarine - monounsaturated, regular
Margarine - monounsaturated, reduced fat
Lard or dripping
h) Olive oil
i) Canola oil
j) Soybean oil
k) Gold'n Canola
l) Other vegetable oil _____

6.3 Fat on Meats/Chicken_____ _____

How much fat is trimmed from meat before cooking/eating?

None
25%
50%
75%
All

How much skin do you eat on chicken?

None
25%
50%
75%
All
Other, please specify: _____
_____

6.4 Salt_____

All the time during cooking
All the time at the table
Some of the time during cooking
Some of the time at the table
Never during cooking
Never at the table
I don't use salt at all

What do you consider to be a serve of salt? _____

Appendix 4 – Intervention documents

A-4.1 Group Allocation Instruction Forms

N-3 Group

the m.o.d.e.l. study
muscle mass, omega-3, diet, exercise and lifestyle

Thanks for participating in 'The MODEL Study': a nutrition and exercise program for women after treatment for breast cancer.

We greatly appreciate your participation in this trial as the information gathered will be used to further the health of breast cancer survivors both in Australia & internationally.

You have been allocated into the following group:

Daily capsule consumption

You will be required to consume capsules containing omega-3 fatty acids for the next 6 months. Below are details of your study commitments for the next 6 months

PLEASE RETURN ALL BOTTLES WHEN YOU HAVE FINISHED THEM (They will be collected at 12 and 24 wk assessments, or in between if convenient)

Ongoing assessments

12 & 24 weeks:	After 12 and 24 weeks, you are asked to repeat the baseline testing that you have just completed.
Important dates:	12 week testing: TBA; 24 week testing: TBA

Capsule consumption

Dose:	**5 capsules per day**
Method of intake:	It is best if the capsules are consumed around the time of any meal, they can be divided into multiple doses throughout the day, or be taken all at one time.
Storage of capsules:	Please keep the capsules in a cool, dry, and dark location. It is appropriate to keep the capsules in the fridge.
Adverse reactions:	If there are any severe effects like swelling in the throat, rash, hives and others please contact your doctor immediately. In addition, please notify research staff as soon as it is convenient for you.

Your supply of capsules

Pick up:	You will be required to pick up a supply of capsules every month over the next 6 months. Your first pick up has occurred already. You will receive notice about when to come and pick up your capsules.
Location:	Level 8, East Wing, The Wesley Hospital, Auchenflower. I.e. Where the initial tests were carried out for the study. Can arrange to do a drive-by pick up.
Miss a session:	If you know you cannot make it in time to refill your capsules please contact Cameron as soon as you are aware of this and other arrangements will be made.

If you have any queries or comments, please contact the study co-ordinator, Cameron McDonald, using the following details:

Ph: 0411380566; Email: UQbreastcancerstudy@gmail.com

Lifestyle Program Groups

You have been allocated into the following group:

the m.o.d.e.l. study
muscle mass, omega 3, diet, exercise and lifestyle

Daily capsule consumption and participation in a specialised nutrition and exercise program

Owing to the nature of this research study, both you and those people guiding you through this intervention do not know if you are to be taking the omega-3 fatty acid capsules, or the placebo (fake omega-3) oil. This will be revealed only after all participants have completed the study.

PLEASE RETURN ALL BOTTLES WHEN YOU HAVE FINISHED THEM (They will be collected at 12 and 24 wk assessments, or in between if convenient)

Below are details of your study commitments for the next 6 months

Capsule consumption

Dose:	5 capsules per day
Method of intake:	It is best if the capsules are consumed around the time of any meal, they can be divided into multiple doses throughout the day, or be taken all at one time.
Storage of capsules:	Please keep the capsules in a cool, dry, and dark location. It is appropriate to keep the capsules in the fridge.
Adverse reactions:	If there are any severe effects like swelling in the throat, rash, hives and others please contact your doctor immediately. In addition, please notify research staff as soon as it is convenient for you.

The 12-week intervention

Attendance: The nutrition and exercise program will have 9 education and exercise sessions starting next week continuing over the next 12 weeks.

Session	1	2	3	4	5	6	7	8	9
Projected dates	31/1/13	TBA	TBA	TBA	TBA	TBA	TBA	TBA	TBA

Duration:	Each session will be 60-75min in duration & will include exercise.
Clothing:	Wear light, comfortable clothing and a supportive sports shoe
Location:	Level 8, East Wing, The Wesley Hospital, Auchenflower. I.e. where the initial tests were carried out for the study.
What to bring:	Pen or pencil and notebook if you would like to make additional notes
Parking:	Will be covered as per normal
Miss a session:	If you know you cannot make a particular session, let Cameron know as soon as possible, and other arrangements will be made. It is hoped you can attend every session.

If you have any queries or comments, please contact the study co-ordinator, Cameron McDonald, using the following details:

Ph: 0411380566; Email: UQbreastcancerstudy@gmail.com

A-4.2 The M.O.D.E.L Lifestyle Program Manual

A-4.2.1 The MODEL Study weekly session summary

the m.o.d.e.l. study

muscle mass, omega-3, diet, exercise and lifestyle

Designed & created by Cameron McDonald

PhD Candidate, 2011

The University Of Queensland
AUSTRALIA

Introduction

Healthy eating habits and exercise are vital components to an optimal recovery after treatment for breast cancer.

The effects of the treatment and other factors place strain on your body, and these strains may be lessened through healthy lifestyle habits.

A diet rich in nutrients, providing adequate energy for the working cells and regular physical activity improve the health of your heart, muscles, brain and immune system. In addition, these habits have been shown to reduce the feelings of fatigue, improve feelings of well-being and help you perform your daily functions.

The MODEL program is one that is designed to improve the lifestyles of women who have completed treatment for breast cancer.

The program is designed to educate you on how to include healthy foods in your diet and how to safely increase your fitness and strength through endurance and resistance exercise training.

Areas of focus for the program

Food and nutrition related

- General healthy eating
- Mindful eating
- Inclusion of fruit and vegetables
- Healthy meat choices
- Identification of foods to reduce
- Breast cancer related nutrition – the latest science on nutrients
- Healthy cooking tips and meal fat reduction

Exercise related content

- Starting and progressing both endurance and strength training programs
- Weekly supervised exercise sessions
- Safety in exercising for risk of lymphoedema
- Increasing physical activity throughout the day
- Learning how to further your own program

How Breast Cancer and it's Treatment Affect the Body

Both diagnosis and treatment of breast cancer take a serious toll on the body and the spirit. Sometimes knowing why your body is behaving the way it is gives you some relief in that, firstly, you are not the only ones experiencing what you are experiencing, secondly, there are things we can do to help!

Below is just a small list of the issues that may affect you after treatment:

Fatigue and Lethargy	Chemotherapy and radiation therapy are known to be taxing on the body, and partially toxic to the muscles. When the muscles are damaged this can lead to a significant amount of fatigue. In addition, it may make you feel like doing less, and this lower level of activity increases the feeling of fatigue – it's a vicious cycle. In particular, Taxane chemotherapies seem to cause significant muscle weakness and tiredness in the leg muscles. Currently, the only way to reduce this is through exercise.
Reduced movement in the arms	The treatment to your lymph nodes can cause what is called 'cording', where it feels like there are thick bands of tissue limiting your movement. This is common, and a physio + regular movement can assist in improving this.
Weight gain and loss of muscle tone	Even when you have been active, and have been careful with your food weight gain is still very common. Currently, it is not completely understood why this weight gain occurs. After treatment for breast cancer, the majority of women put on weight even when being careful with their food intake. A combined approach of exercise and diet is key.
Lasting effects on your metabolism	Breast cancer and it's treatments can cause a change in your body in regards to heart disease and diabetes. If these changes are left unchecked, it can result in a faster development time of these conditions. A healthy lifestyle is essential for reducing these changes, and therefore lowering your risk of those diseases.

Participating in regular exercise, while it may seem difficult at the start, is one of the only ways we know that will reduce fatigue, prevent muscle loss, improve arm mobility, and improve your metabolism. Healthy eating is an important consideration in maximising the effects of exercise, and should be considered essential for a long post-treatment period.

Table of Contents

Week 1 - Introduction to healthy eating, exercise & stretching

The purpose of the first session is to help you understand the overarching principles of healthy eating and exercise.

It is understood that a large number of people know the majority of this information, however we often do not put it into practice.

There are certain nutrients that your body needs, and a certain amount of physical activity that will greatly improve your health. Just by increasing the consumption of these foods, and performing some exercise above what you are doing currently will improve your health.

Use the following as a checklist of things, and try and achieve one or two more things each week

Basic Healthy Food Choices Checklist

- 2 pieces of Fruit per day
- 2 meals containing vegetables
- Wholegrain choices
- Handful of nuts
- Lower fat dairy options
- Lean meat choices with the inclusion of meat alternatives
- Enjoy extra foods mindfully
- Alcohol in moderation

- Increased energy levels
- Maintenance of healthy weight
- Healthy blood pressure, sugar levels, cholesterol
- Better recovery from exercise

Exercise & Movement

- 3 exercise sessions this week
- 2 Aerobic sessions
- At least 1 resistance session

- Muscle mass, strength, power
- Cardio-respiratory fitness
- Better sleep
- Physical activity levels
- Flexibility
- Improves your immune system
- Body image, self-esteem and mood

Tactics for moving more

Active transport

-Get off the bus a stop earlier

-Take the stairs instead of the lift

-Park the car further away at the supermarket

Active Excuses

-Throw away the TV remote control

-Enjoy a walking lunch

-Take the dog for a walk

-Meet up for a walk and a coffee

-Walk the children or grandchildren to school

Week 1 Activity: Your food checklist

Over the next week, start taking note of the choices you are making with your food. Use this practical activity as a starting point that will increase your awareness, and guide you as to which foods to include more of.

Tick it off if you have feel you have reached the goal for each of the foods on the checklist.

Day	Fruit 2 piece	Veg 5 serve	>75% Wholegrain	Low fat dairy	Lean meat	Nuts	Legumes
1							
2							
3							
4							
5							
6							
7							

Your Exercise checklist

Day	1	2	3	4	5	6	7
Walk							
GymStick training							

Goal Setting

Without direction or a destination, we don't know where we are going and we don't know it when we get there!

Any time you want to change your habits for the long term you have to have a very clear reason as to **why** you are wanting to change. We all know the **what**, i.e. to get fitter you need to exercise more, however, **why** is improved fitness important to you?

If you can start to visualise how you will feel when you reach your goal, this is a really good way of building something to strive for. If you don't have that vision or that emotion pulling you towards it like a big unstoppable magnet, then there's a chance the change will seem too hard.

Use the following exercise to combine both the **What and the Why**, and keep these things in the forefront of your mind when you are going through this program

Be **S**pecific, **M**easurable, **A**ccountable, **R**ealistic, **T**ime oriented

Goal 1 – Short term				
Push ups	Perform at least 15 in one go (all the way to the ground)	Record all sets of push ups in my journal	Achieve it by the end of January	Where am I now? 5 push ups.

Why is this goal important?

If I can improve my push ups it will give me the confidence to engage in more difficult activities that I have been wary of. In addition, I want to feel like I am in charge of my strength, and completing this will mean I have some control over my body.

What does

Goal 2 – Long term				
Improve my energy levels	I want to feel like a 8-10 out of 10 when I wake up and start the day and have energy all day	Record my energy levels through the day	Achieve it within the next 6 months	Where am I now? Feeling like a 5 or less

Why is this goal important?

My energy levels is one of the things that was reduced after treatment. When I get my energy levels back it will mean that I am able to work harder, take better care of the family and feel like my old self (or even better than that!). When I have better energy levels it will allow me to feel like I have some control of my body that was reduced during treatment.

Just some examples...next, it's your turn. Please write down as many goals as you like. Paint the picture of what it will be like when you achieve your goals.

Goal Setting - Practical

Be **S**pecific, **M**easurable, **A**ccountable, **R**ealistic, **T**ime oriented

Goal				

Why is this goal important?

Goal				

Why is this goal important?

Goal				

Why is this goal important?

Goal				

Why is this goal important?

Week 2 – Eating more plant food

Fruits, vegetables, nuts, seeds and legumes are amongst the most important foods you can consume for overall health and well-being. Thinking about these foods as something that your body needs every day is very important.

Use some of the tips below to use vegetables and fruit more commonly in your daily meals

How can I eat more vegetables?

- Try to ensure that half your dinner plate is vegetables or salad by serving 2-3 types of vegetables with your main meal or serving vegetables and a side salad
- Whenever you see a dish that has a sauce component, i.e. mince, soups, stir-fry or casserole – ***get into the habit of adding lots of vegetables to bulk it up.***
- Add extra salad to sandwiches and rolls or perhaps serve salad alongside a sandwich
- Spend 30 minutes on the weekend to roast some vegetables, e.g. onion, sweet potato, capsicum, carrot, swede, turnip, pumpkin – enjoy the change in flavour
- Use tinned/ fresh tomatoes as the base for a pasta or rice dish
- Serve crunchy raw vegetables (i.e. carrots, celery) as a snack
- Used chopped or puree vegetables to make a low fat sauce
- Add extra taste to cooked vegetables with low fat flavourings such as herbs, spices, lemon or orange juice, pepper, garlic, chili or chopped fruit

How can I eat more fruit?

- Try adding fresh, frozen or dried fruit to breakfast cereals
- Blend soft fruit like bananas and strawberries with skimmed or reduced fat milk or plain low fat yoghurt for a smoothie. You could also try freezing smoothies in ice block moulds.
- Eat fruit as snacks between meals & keep fruit available in a fruit bowl
- Add fruit like apple, pears, peach and grapes to salads
- Top toast or muffins with slices of fruit e.g. bananas
- Choose fresh, canned (in natural juice) or stewed fruit for dessert
- Prepare stuffing for meat, fish and poultry dishes from fresh or dried fruit
- Try having a glass of unsweetened fruit juice (fruit juice should only count for one serve of fruit/vegetables a day)

Consuming more legumes

Anything that has a sauce involved is perfect for the addition of legumes. They are cheap, fibrous, nutritious and don't really affect the flavour of the dish.

- Add lentils or beans to bolognaise, lasagna or chili con carne

- Add beans, lentils or chickpeas to a stir-fry

- Add any or all to curries, soups, casseroles, stews

- Home-made bean dishes: Diced tomatoes, five-bean mix, extra virgin olive oil, garlic, onion, mixed herbs, +/- a touch of chili and simmer for 5 minutes.

If you like them:

- Add them to a salad for a great colour and texture change!

Making the most of shopping

Meal Planning and Fruit & Vegetables

Food	Amount	Options
Fruit	2 pieces per day per person.	Fresh fruit Frozen fruit: mango, berries etc Canned in juice Dried fruit and/or nuts
Vegetables	6 types of vegetables per day	**Always lots of green**: Broccoli, Spinach, Rocket, Celery, Lettuce etc. **Then at least 3 other colours**: purple cabbage, onion; yellow/orange capsicum, pumpkin, sweet potato carrots; red capsicum, tomatoes; & many others. **Frozen vegetables** are great for regular consumption or if you need a quick fix
Legumes	75g per day per head	Get a mixture of big and small cans for whole meals and individual meals alike. Canned is ok. Fresh are good and require soaking. - Cooking: normally 45-60 minutes on simmer, perfect for a curry/soup/casserole/stew that is cooking for a while.
Nuts	Handful each day	Raw and unsalted is always best. If you love salt – add a salted nut to a mixture of unsalted nuts. Toasted nuts are ok.

Shopping Tips

- Plan your menu in advance as much as possible
- Buy fruit and vegetables in season
- Plan your menu around the specials for that week (fresh produce that is on special is on average 35% cheaper than normal price)
- Include more fruit and vegetables and less meat in the menu plan. Meat is almost always more expensive per kilogram than fruit and vegetables
- Buy less pre-packaged items (for example chopping your own coleslaw ingredients rather than buying them pre-chopped)
- Offering fruit and vegetable snacks for morning and afternoon snacks rather than expensive packaged snacks such as muesli bars and fruit rollups
- Look at the cost for weight and compare value for money. For example a 250 gram pack of chopped mushrooms may only be $3.00, but if the cost of loose mushrooms is only $6.99/kg then the pack becomes very expensive by comparison (250g x 4 = $12.00 per kilogram)
- Avoid shopping when hungry
- Buy in bulk where available and practical. However, ensure that you have sufficient space to store the bulk items under the right conditions to prevent them from spoiling
- Consider buying marked down fruit and vegetables if they are still in a reasonable condition.

A budget consideration

By replacing mince in recipes with vegetables or lentils you save a considerable amount of money per meal. Or you can use your original recipe and add the legumes to make the whole meal go further for cheaper!

Mince: $10-$14/kg; Lentils and legumes: $3-$4/kg.

By removing ¼ - 1/3 of the mince from a recipe you stand to save close to $3 per meal. Over the month that can be an extra $80 in the pocket + you are getting healthier.

Great vegetables to grate and add to a mince dish or one with sauce:

- Zucchini, carrot, pumpkin, swede, turnip, sweet potato

Week 3 – Healthy weight, Mindfulness and First Serve Portion

Key points for managing a healthy weight…

1. Always have breakfast, or food within a couple of hours of rising.

2. Choose nutritious foods to abate hunger – including snacks

3. Consume water before and during every meal – aim for 2L per day

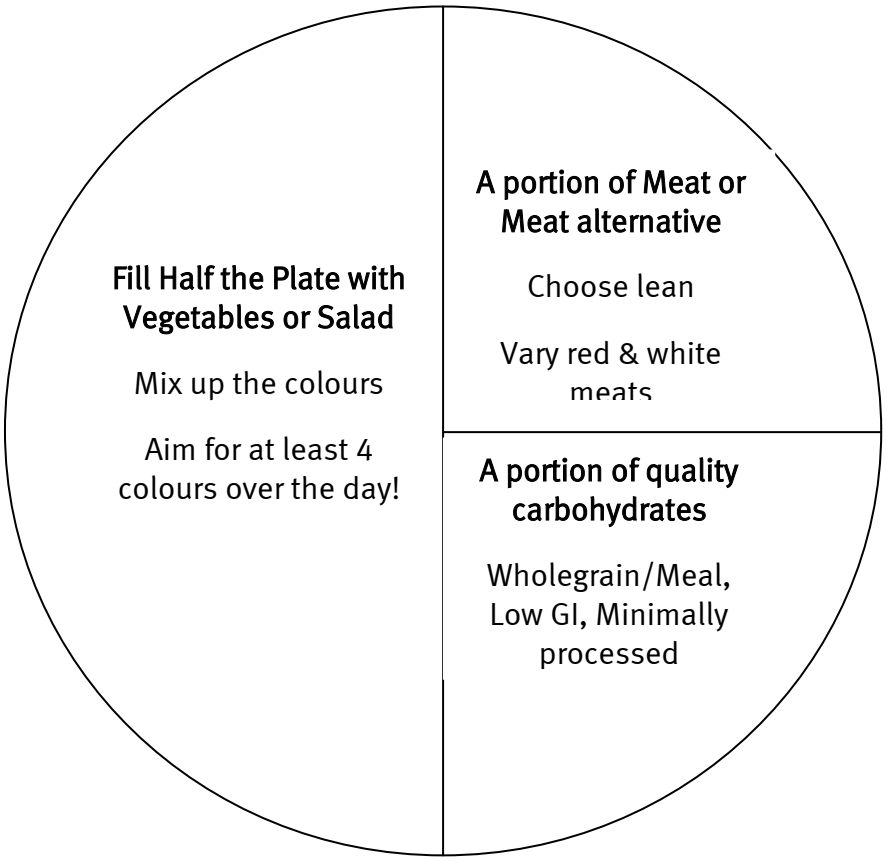
4. Have at least 3 meals each day that contain some protein, healthy carbs, healthy fats and plenty of fibre.

Maintaining a healthy weight improves the health of your heart, enables you to better control blood sugar levels, takes strain of your joints, improves mobility and function.

In addition, energy levels are normally better and self-esteem often improves.

First plate portion

A great way of ensuring you receive all your key nutrients in a portion that is suitable for your hunger levels and appetite.



MINDFUL EATING – Having what you want, when you feel like it.

It's time to enjoy the foods you love without the guilt!

Mindful eating is a process where you learn to maximally enjoy your foods. It will help you consume less high fat and high sugar foods, without letting you feel deprived of all the good things in life!
The principles of mindful eating are very simple, firstly, it is important we are putting the right type and amount of nutrients in:

1. **Your body needs wholesome foods in order to operate**
2. **When you are hungry, your body is asking for nutrients**
3. **We eat when our body tells us we are hungry, we should listen to it!**
4. **When we are no longer hungry the body doesn't need more food**

The great thing about mindful eating is that if you feel like a treat or some sweet/savoury foods, YOU CAN HAVE IT!

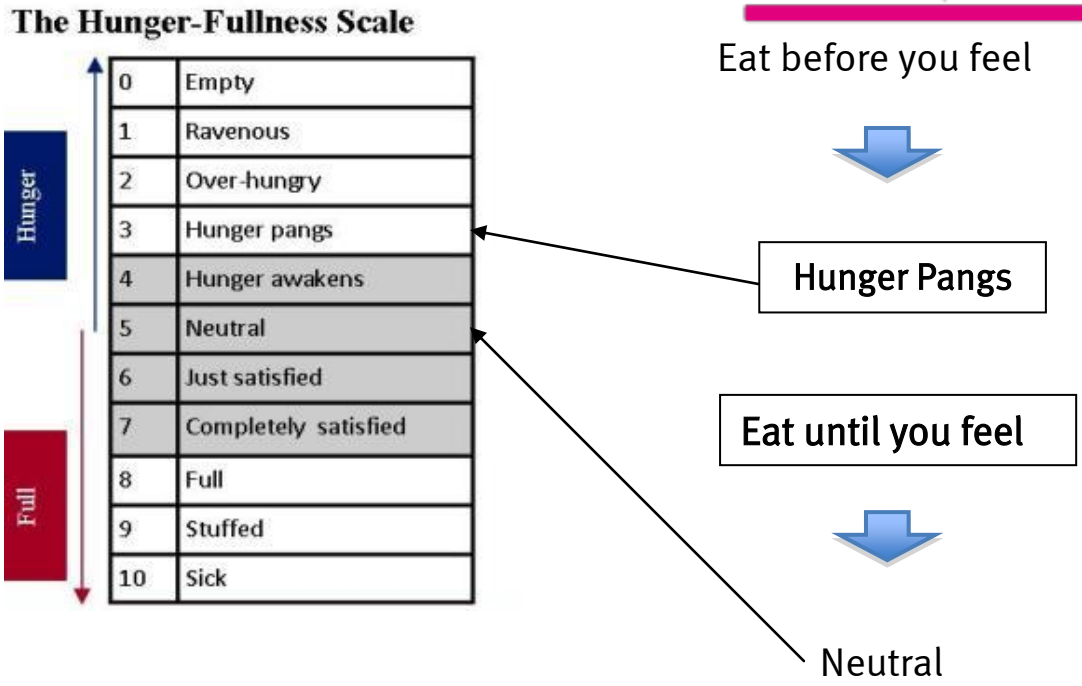
'But first ask yourself, 'Am I really hungry for this food?'

Often we have high fat or high sugar foods out of habit, plus there is an element of liberation and enjoyment that comes along with these foods. However, what are the purposes of these foods? We know that the wholesome foods listed above are filling our body with the nutrients we need for good daily function, so the only purpose of these treat foods are flavour!!!

Once high fat and sugar foods have passed the tongue, there is no more purpose for it. Sooo, it's very important that we taste these delicious foods for as long as possible before swallowing. Follow this step-by-step guide to really getting the most out of your sweet foods.

1. Remove distractions while you are eating – e.g. watching TV, driving, cleaning up etc are all ways to decrease your ability to taste.
   -Grab a chair and focus on the wonderful food you're about to eat!
2. Use all your senses – look at it, smell it, touch it before you taste it – this is a guaranteed way to make the memory of this food last for a lot longer.
3. Take a small piece of the food and taste it for at least 2 minutes. Roll it round, break it down and try and pick the flavours – this will maximise your enjoyment of the food and make you aware of every flavour in it.
4. Ask yourself after every bite – 'Do I feel like more?'
   Often, after only a few bites of chocolate, the next piece doesn't add to your enjoyment anymore. It's at this time that you might consider putting the rest away, knowing that you can have more if you feel like it later.

These principles are about enjoying the foods that are designed to taste wonderful. Listen to your body – it does know when you need food! Feel liberated in having your favourite foods when you desire, and know that when you have them, you will enjoy them maximally.



After your meal: *Take 15-20 minutes and then decide if you want more!*

Making the most of mindfulness!

What can help you maximize your mindfulness is to plan some treats into your week. Knowing that you can have them enables you to make an objective decision about whether or not you feel like it, AND allows you to have it without the guilt.

List your favourite foods – **the ones you can't live without,** and plan them into your week.

My favourite foods are…	**Day & Time to have them**
E.g. Chocolate bar	*Monday morning tea*

You may discover that you don't like these foods as much as you used to – in that case, it is ok to not eat them!

Week 4 – Eating the 'right fats'

Lowering saturated fat intake

Sources of Saturated Fats	Lower fat substitutes
Animal meats Fresh meats (white) Processed meats ++	- Trim the fat off the meat - Choose lean cuts (‹5% fat) - Limit intake of processed meats
Dairy foods Double, thickened, pouring cream, Creme fraiche Sour cream, light sour cream Butter, Lard, Dripping Cheese	- Evaporated milk (Carnation) Extra light sour cream, Natural yoghurt Ricotta, Cottage cheese - Margarine, lower fat butter, unsaturated cooking oil. - Small portion, cream cheese, low fat
Extra Foods Pastries Commercial cakes Deep fried foods Chocolate Chips Coconut cream	Limit intake of these foods through mindfulness and more nutritious snack selections - Evaporated milk + coconut essence

Ways to increase Poly and Monounsaturated fat intake

1. A daily serve of nuts (particularly walnuts, brazil and pine nuts)
2. Healthy cooking oils: Sunflower oil, rice bran oil, grapeseed oil
3. Healthy dressing oils: Extra virgin olive oil
3. Consume specific plant foods: Olives, Avocado, Seeds, Nuts & Olives.
4. Using a polyunsaturated margarine (Nuttelex, Meadow Lea)

Healthy cooking methods

Cooking Method	It's advantages/Methods
Grilling	- Will allow fat to drain away - Fast, convenient and gives great flavour Be careful of: - Cooking at high temperatures and charring the meats
Steam/ Microwaving	- Fast, convenient - No problems with charring or burning - Best for fish or chicken ○ Use of herbs/spices is a good idea
Boiling	- Removes most of the fat from the meat - Great way to achieve moist meat - Use stock/herbs/spices in the broth to add flavour ○ After boiling, throw into the grill or fryer to give it a nice crusty edge.
Baking	- Great way to give the same foods a different flavour: carrots, sweet potato, pumpkin - Use a baking tray & water instead of oil. Brush the oil on to prevent drying of meat/vegetables
Frying/BBQ	- Use a pan that enables fat to drain away - Don't allow food to char - Seal meats either side then turn them more frequently

Additional Tips for healthy fat consumption

Great foods to add in:

Linseed, Sunflower & Almond meal – Cereal, smoothies, yoghurt

Mixed seeds and grains – healthy section at supermarket (pepitas, sunflower, flaxseed/linsseds, chia seeds and others)

Use marinades to maintain tenderness & protect the foods

Use herbs and spices to increase flavour without the need for added fats

Allow meat to rest for 5 minutes after cooking

Place in a bowl covered in al-foil and put in oven @ 100^{0}C for 5 minutes

Week 5 – Meat and salt intake

Meat and Meat Alternatives

Meats and alternatives are vital for adequate intake of protein, some fats, essential vitamins and trace minerals. You do not need large portions of these foods to maximize their benefit

1. Aim for a variety of meats each week
2. Choose lean, unprocessed meats
3. Consume red meat in moderation
4. Reduce the charring from high cooking temperatures, especially BBQ meats.
5. Try vegetable based protein sources

    - Legumes: chick peas, lentils, beans;
    Soy based foods: tofu, tempeh; &
    Nuts and Seeds.

Salt Consumption

A high salt intake is not beneficial for health, and may be related to an increase risk of stomach cancer and high blood pressure. Most salt in our diet is within the foods we eat, while the rest is added salt during or after cooking. It is important to reduce salt intake and increase the consumption of foods that contain potassium, calcium and other minerals.

1. Choose lower salt varieties of canned foods, stock cubes
2. Limit obviously salty foods
3. Use herbs and spices to cooking for flavour
4. Eat fresh foods, as opposed to processed foods
5. Allow 4 weeks for your taste buds to adjust to the lower salt intake
6. Compare labels for: biscuits, canned foods, chips, sauces, and cereals.

NUTRITION INFORMATION

Servings per package: 3
Serving size: 150 g

	Quantity per serving	Quantity per 100 g
Energy	608 kJ	405 kJ
Protein	4.2 g	2.8 g
Fat, total	7.4 g	4.9 g
– saturated	4.5 g	3.0 g
Carbohydrate, total	18.6 g	12.4 g
– sugars	18.6 g	12.4 g
Sodium	90 mg	60 mg
Calcium	300 mg (38%)*	200 mg

* Percentage of recommended dietary intake

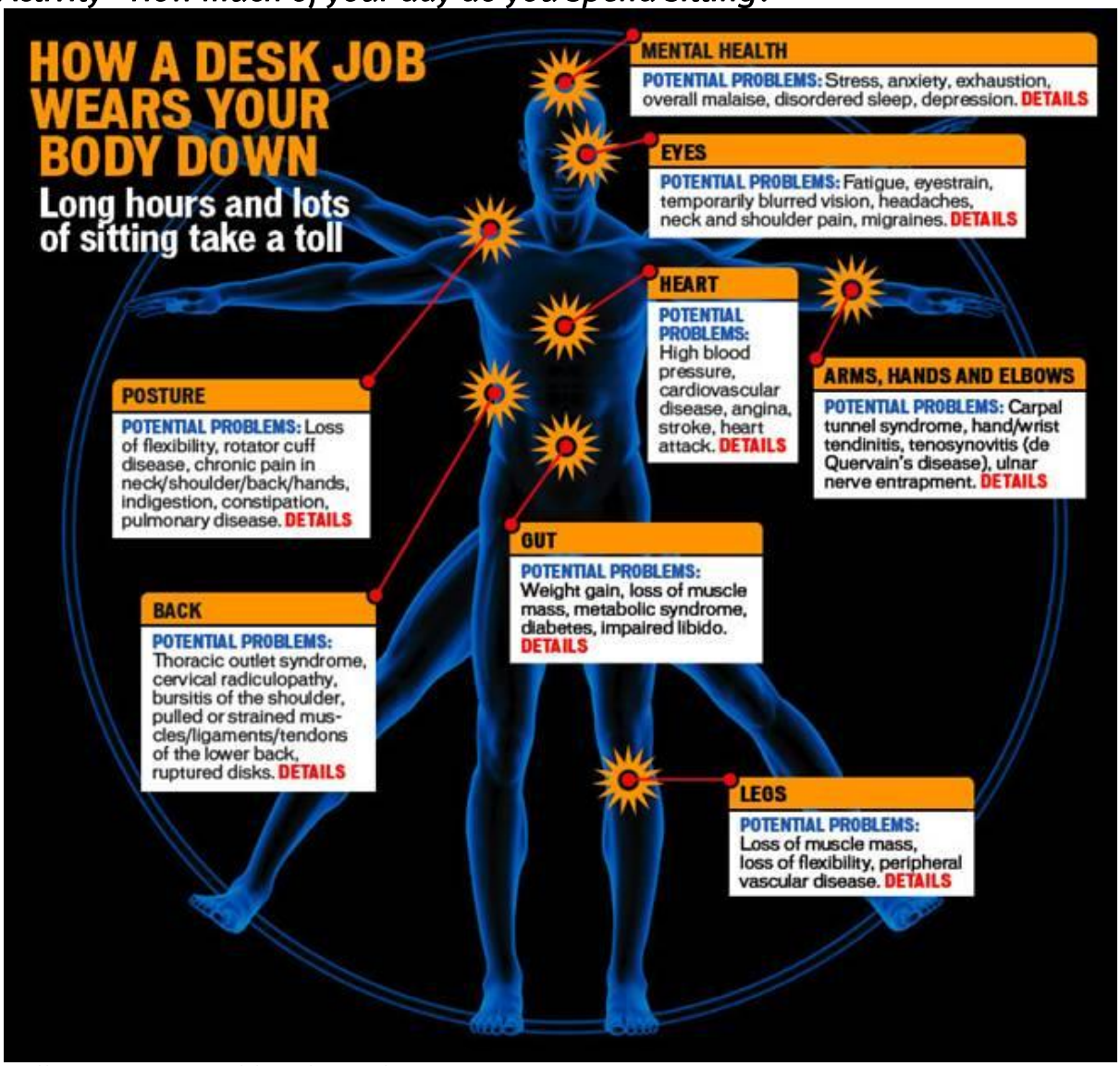
Ingredients: Whole milk, concentrated skim milk sugar, strawberries (9%), gelatine, culture, thickener (1442).

Sedentary activity

Record the time you spend sitting on an average day, and how long this sitting is uninterrupted

Activity	Total time sitting	Sitting without a break
Watching TV		
At work		
Transport		
Meal Times		
Coffee shops		

Activity - How much of your day do you spend sitting?



http://www.computerworld.com/s/article/9115340/Health_hazards_for_IT_workers_how_that_desk_job_wears_your_body_down

Tactics to decrease your sedentary activity

1. Know that sitting for longer than 30 minutes without a break has a negative impact on your health – it reduces fat burning, decreases uptake of blood sugar, may add to fat stores around your abdomen and puts strain on your heart.

2. Think of the times you are sitting still and think of how you may increase movement at those times:

Seated activity	Options to reduce sitting time
Watching TV	- Use the ads as an excuse to move: Grab a glass of water, do a few sit to stands, do some cleaning, just stand up - Exercise during your favourite shows. TV doesn't often require a whole lot of your attention, so perform some of your GymStick or body weight exercises while you watch. - Reduce the amount of TV you watch.
Sitting at work	- Phone call/email rise: Stand up and squat whenever you get a phone call/email - Talk to people in person, as opposed to calling them - Put a box on your desk and work standing up! (Sometimes not practical) - Take a walking morning tea break with your drink or snack.
Driving in the car Sitting on the bus	- Practice your core/foundation exercises in the car: Shoulder blades back and down, core muscles on, chin tucked. - Flex your gluteus (bottom), legs, arms, shoulders and neck muscles as you drive – muscle activation counts! - Ride a bike or walk to work where possible - Take a stop off each end of the bus and walk further - Occupy a standing space on the bus

Decreasing your sitting time is always going to be different when comparing different people.

The essential component is that if you move, then your body wants you to hold onto the tissues that enable you to move, i.e. your muscles.

If you know you have been sitting for a long time, then try standing up, moving around and see how different you feel afterwards. It should give you some energy back and reduce fatigue.

It all starts with your awareness!

Week 6 – Alcohol, Drinks and Socialising

Appropriate Alcohol Consumption

Is it good for you?

Often it is stated that 1-2 standard drinks can be beneficial for heart health. However, it is very important to note that while some benefits may exist for heart health, alcohol may be harmful to your breast cancer related health even at small levels of consumption.

For women who have completed treatment for breast cancer, reducing alcohol consumption to the safe drinking levels is very important.

Alcohol in moderation is still key, and no-one should increase their consumption to achieve health benefits.

"The safest range of alcoholic consumption is between 0 & 1.5 standard drinks per day."

Full – 5%
1 std = **285ml**

Mid – 3.5%
1 std = **375ml**

Light – 2.7%
1 std = **570ml**

High – 14%
1 std = **90ml**

Normal – 12%
1 std = **100ml**

Low – 9-10%
1 std = **125ml**

Spirits – 40%
1 std = 30ml

Fortified – 20%
1 std = **40ml**

Liquers– 20%
1 std = **40ml**

See above for strengths of different alcoholic beverages and the amount that relates to one standard drink

Tips for reducing your alcohol intake…

1. Switch to standard sized drinks (smaller) and sip slowly
2. Add ice
3. Enjoy a wine spritzer
4. Switch to light beer
5. Alternate alcohol with water
6. Don't refill your glass until it is empty
7. Reduce the number of days you have a drink
8. Apply mindfulness to your drinking

Energy from Beverages

Beverages including juices, soft drinks, flavoured milks and alcoholic beverages contain a considerable amount of energy (kilojoules). The problem is that these fluids will not often give us a feeling of fullness that matches the energy they are providing.

Juices – as good as fruit?

Fruit juices that contain often contain a number of beneficial nutrients, however they normally contain a higher concentration of carbohydrate that found in whole fruits. In addition, they contain very little fibre, which is one of the more beneficial components of fruit consumption.

1. Always choose a piece of fresh fruit over its juice. Replace the juice with water.

2. When consuming juice, aim to have fresh juice as much as possible and add the pulp back into the juice (good source of fibre)

3. Avoid fruit **drink** – typically these have very little real fruit in them.

Soft drinks

There are no benefits to be received from consuming soft drinks. Therefore, enjoy them mindfully and look for options like water, weak cordial or juices as preferred beverages.

Regardless of full sugar or diet soft drinks, these beverages should be consumed on an occasional basis. If your current consumption is large, then switching to a diet variety while you try to reduce your intake is an option for you.

Flavoured milks

Flavoured milks typically are quite high in energy (300ml = 900kJ), and therefore going for a light version, or lower sugar option is beneficial.

After exercise, particularly if you have been exercising intensely for over an hour, a flavoured milk is a fantastic recovery drink that has enough protein and carbohydrate to maximize your recovery. **However, a normal nutritious meal will also allow you to recover as effectively.**

The drink 'kilojoule-o-meter'

0kJ 50kJ 100kJ 200kJ 300kJ 400kJ 500kJ 600kJ 700kJ 800kJ 900kJ 1000kJ

‹100kJ	‹400kJ	‹700kJ	›700kJ
Water	Skim Coffee/Milk	250ml Whole milk	Soft drink – 500ml
Tea (+/- milk)	300ml Vegie Juice	400ml Juice - Natural	Juice – 500ml
Coffee (black)	*1 std drink: wine, lite/mid beer, nip spirits*	*1 Bottle full strength beer*	Milk (choc…) – 300ml
Diet drinks			*Alco-pops – bottle*

Socialising with awareness

It is part of our culture to enjoy foods with friends at social functions/gatherings. It is often at these times that we consume a considerable amount of food that although tastes great, has a high level of fat and/or sugar. Particularly during the festive season, and at other times when we are socialising regularly, the foods at these functions can contribute considerably to our intake.

A couple of guidelines:

1. If it is a one-off event and it is not happening regularly – DON'T WORRY ABOUT IT! If you were to hinge your health goals on one night out, then we are blaming the mouse for sinking the boat!

2. If it is a regular social function or you are looking to reduce your intake of these foods (which never hurts), then read the tactics below for decreasing your consumption.

For functions providing finger food

- Have a full nutritious meal before you go to the function – abates hunger.
- Don't stand next to the food platter – this will reduce grazing.
- Drink plenty of water – keeps your mouth busy!
- Be selective about the foods you want to try – really focus on the food and experience the flavour.
- Be mindful of how full you are – you can always have more if you feel hungry.
- Provide a platter of vegie sticks, healthy dips & fruit.

When attending a buffet

- Employ the first portion rule – plenty of vegetables and salad, a serve of meat and pasta/potato/rice
- Ask yourself – Do you really feel like it? Or are you just feeling like you're hungry because it is in-front of your eyes?
- Share a plate with a friend to get more variety while keeping the amount the same
- DON'T RUSH: Consume it slowly, take time to taste it, give yourself time to feel full before rushing back and loading the plate up again

Week 7 Handout – Food Labels

The nutrition label reading should be used to assess the best food according to: fat, sodium, fibre and in some cases, sugar content.

However, food groups will vary widely and types of food in specific products will have a large impact on the nutrition label.

Per 100g	Breads + Cereals	Dairy Foods & Cream	Snack foods	Canned foods & sauces
Energy	N/A	N/A	**300-500kJ/ serve**	N/A
Protein	N/A			
Total fat	Saturated ‹33% of Total Fat	**‹3%** for milk & yoghurt **‹7%** for cream, sour cream, evaporated milk Cheese – portion size not fat content	**No limit** Nuts could be 60-90g fat per 100g	**No limit**
-Saturated Fat		*Will always be 60% saturated*	Saturated ‹33% of Total Fat	
Carbohydrates	**No Limit** Will often be 50-70%	›10g per 100g indicates added sugar	**No Limit** provided energy and type of fat is looked after	**No Limit**
-*Simple sugars*	If cereals contain fruit: can be up to 20g per 100g Higher for: baked, toasted, added sugar. Oats contain ‹1g/100g	‹15g per 100g Better to have ‹10g/100g		Canned tomato products often contain added sugar – check ingredients list
Sodium	**Not vital** Breads: ‹500mg Pasta: ‹120mg Cereals: ‹200mg *All Bran = 650mg, ok as an addition*	**N/A**	Compare for chips, savoury biscuits, dips. Use total energy as guide for how many you eat	Aim for ‹120mg Or No added salt varieties
Fibre	**›7g per 100g** ***Even ›10g is a good aim***	**N/A**	N/A	N/A

All fruits, vegetables and lean meats can be considered appropriate for consumption, and will not often contain a nutrition label. Processed meats and packaged fruits (two fruits etc) are worth comparing though

Foods are not defined by one bad nutrient

Breads and cereals

- Typically high in sodium compared to fruit and vegetables, however benefits come from fibre, vitamin, mineral and antioxidant content.
- High sugar content may be due to fruit – this is still ok!

Dairy foods

- Always have a high percentage saturated fat of total fat.
- High in protein, and vital vitamins and minerals. Milk normally has low absolute content of fat (‹4%), and satisfies hunger well.
- For foods like cream, sour cream, thickened cream & butter, comparing fat content of their 'lite' versions can greatly reduce your fat intake.

Snack foods

- Unless they are healthy options like fruit, vegetable sticks, glass milk & nuts, it is good to aim for around 300-500kJ per snack
- To really optimize snacks, compare saturated fat, sodium & sugars, however the main aim is to reduce the energy of the snacks

Canned foods and sauces

- Typically high in salt – look at the sodium/100g as the main focus.
- Some sauces have a large amount of added sugar – compare products within the same range.

Understanding words on the ingredients list

Sugar	Fat	Salt
▪ Dextrose ▪ Disaccharides ▪ Fructose ▪ Glucose ▪ Golden Syrup ▪ Honey ▪ Lactose ▪ Malt, Maltose, Maltitol ▪ Maple syrup ▪ Monosaccharides ▪ Sucrose	▪ Animal Fat/Oil ▪ Beef Fat ▪ Butter ▪ Chocolate ▪ Coconut, Coconut Oil, Copha ▪ Cream, Sour Cream ▪ Mayonnaise ▪ Vegetable Oil/Fat ▪ Diglycerides ▪ Monoglycerides ▪ Dripping, Lard ▪ Milk Solids ▪ Oil, Palm Oil ▪ Shortening	▪ Baking Soda ▪ Boosters ▪ Celery Salt ▪ Garlic Salt ▪ Meat/Yeast Extract ▪ Monosodium Glutamate (MSG) ▪ Onion Salt ▪ Vegetable Salt ▪ Sea Salt ▪ Rock Salt ▪ Sodium ▪ Sodium Ascorbate ▪ Sodium Bicarbonate ▪ Sodium Lactate ▪ Sodium Nitrate/Nitrite ▪ Stock Cubes

EXTRA MATERIALS

Includes:

Your training diary

Healthy eating and exercise tips

Safety during training

Measuring your training

Nutrition and Exercise – How they work together

Consumption of specific nutrients around exercise can greatly influence how effective that exercise is for overall muscle health.

Protein and carbohydrates are important nutrients in the hour following exercise. A protein shake is not completely necessary as adequate protein consumption can be achieved through foods like meat, eggs, milk, nuts and seeds.

In the hour following exercise your muscles are primed for uptake of **protein & carbohydrates**, and if these two nutrients are provided, your recovery will be greatly improved.

'Bricks don't lay themselves'. You need carbs to power the protein building process. Just like you need bricklayers to provide the power to the bricks (protein).

Ideal snacks or meals following exercise

Protein – 15 to 25g is required for maximal muscle recovery after exercise

Carbohydrate

- 30g of carbohydrate is needed to refuel muscles and power protein building.

What foods have enough protein and carbohydrates for a good recovery?

Protein: 15-25g	Carbs: 30g	Protein & Carbs
-60-80g meat or Tofu -2-3 eggs -80g cheese (have 40g with some meat)	-2 slices bread; 3-4 rice cakes -2 pieces of fruit or 1 big banana -1 cup pasta, cereal, potato -400ml Gatorade; 300ml juice;	-Up & Go Energise (250ml) -Meat sandwich -Cereal and Milk/Yoghurt -Meat + potato/pasta/rice -Handful of nuts + Yoghurt OR Milk instead of yoghurt -A normal healthy main meal as previously discussed

Plan your exercise to occur just before a main meal.

You can see from the table above that normal meals will accommodate your requirements after exercise, provided it is a balanced meal.

Exercising Safely and Effectively

Always Warm Up & Cool Down – aim for 2-3 minutes

- Warming up prepares the body for exercise and helps to prevent injury and prolong your workout

- Warming up increases blood flow through your muscles and other tissues gradually – cool down helps to help remove excess fluid from your active muscles after exercise.

Both warm up and cool down are essential to help in preventing issues related to lymphoedema and fluid build up.

- A warm-up incorporates both general movements – walking and dynamic stretching + more specific movements that relate to the exercise that follows.

e.g. Before resistance training – walking and stretching the whole body for 90 seconds is a general warm up. Performing an 'easy' or 'light' set of chest press before you try for a harder weight is a specific warm-up to your activity.

Measuring Intensity of your workout

Perceived Exertion

The Borg Scale – a rating from 6 to 20.
It relates to how hard the exercise feels to you.
Rate your perceived exertion on how you feel.
Aim to keep it within 11-14

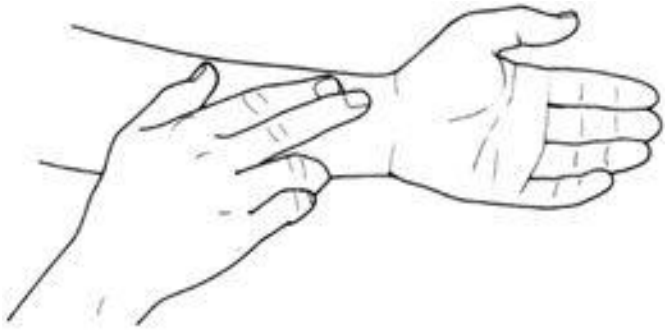
6	No exertion at all
7	
	Extremely light
8	
9	Very Light
10	
11	Light
12	
13	Somewhat hard
14	
15	Hard Heavy
16	
17	Very Hard
18	
19	Extremely Hard
20	Maximal Exertion

Measuring Heart Rate

Find your radial pulse as per the picture below.
Count the number of beats in 15 minutes.
Multiply that number by 4 to calculate beats per minute (bpm).

This is not considered the most accurate heart rate measure, however, it gives you some indication of your heart rate.

Find out what your optimal heart rate is from your fitness results.



Resistance training safety and progression

Safety

- **If you have a lymphoedema garment – WEAR IT at all times during warm up, exercise and cool down.**
- Always perform you specific limb warm up movements before any exercise.
- Always perform shoulder mobility exercises before and after you do any exercise.
- **Technique before power** – All exercises should be able to be completed with correct technique before any progression in weight occurs.
- **Core strength** – before any exercise, think: CORE MUSCLES ON
- Always continue breathing through the exercise
    + Breath OUT when you are overcoming the resistance; breath IN during the opposite movement.
- If in doubt about the technique– wait until you are supervised before attempting the movement.
- INJURY – Please report the injury to Cam ASAP so we can arrange optimal treatment for you – and allow you to continue other exercises.
- Make sure you complete all the exercises – they are designed to bring balance to your muscles – skipping a couple may create an imbalance

Progressing your training

- Once you have completed 10 repetitions for 2 full sets, in 2 consecutive sessions, your program can be advanced.
- Aim for 8-12 repetitions
- All program advancements will be directed by the exercise physiologist staff.
- Progression that is too fast may result in injury or poor technique habits.
- Monitoring your training: Always write down the number of full repetitions completed for each set, for each exercise – your goal is to maintain or improve this number each following set.

Exercise/Date	11/09/2011		13/09/2011	
	Load/Resistance	Reps per set	Load/Resistance	Reps per set
Chest Press	2 Wind-ups OR	1 x 12 1 x 10	3 Wind ups	1 x 8 1 x 7

Aerobic Exercise - Safety and Progression

Safety

- Ensure you have comfortable clothes and supportive shoes.
- Consume water before exercise – take a bottle with you if it is going to be longer than 25 minutes.
- Always start the first 3 minutes with an easy 'warm-up' pace, and finish with a couple of minutes of cool down.
- You should be able to talk – but not sing, or stay within the 12-14 range on the Borg Scale.
- It is good to let someone know when you will be going, or take a friend along with you.
- Be sun-safe
- Wear a hat in cold weather to reduce heat loss.
- Monitor small niggling injuries – if they are getting worse over time – notify study staff ASAP for appropriate management.
- Always think about you core muscles during exercise – protection of spine is crucial

Progressing your training

- Monitor the distance/time and the exertion of the exercise.
- Measure the length of your walking track.
- Always look to increase time OR intensity, not both together

E.g. Your first walk went for 20 minutes and you ranked it as 11-12 on the Borg Scale. To progress your walk you have two options:

1. Increase the time of the walk (+ 5 minutes) and continue at the same intensity
2. Increase the intensity (12-13 on the Borg Scale) and maintain the length of the walk.

DO NOT increase time and intensity together.

After you have increased one component – you can then increase the other for further progression.

Record your progress

Exercise/Date	11/09/2011		13/09/2011	
	Distance + Time	Borg Rating or Heart Rate	Distance + Time	Borg Rating or Heart Rate
	3.3km 25:25 minutes	11-12 110bpm	3.3km in 24 minutes	13 125bpm

A-4.2.2 The MODEL Study Exercise Prescription Manual

the m.o.d.e.l. study

muscle mass, omega-3, diet, exercise and lifestyle

Designed & created by Cameron McDonald

PhD Candidate, 2011

THE UNIVERSITY OF QUEENSLAND
AUSTRALIA

Resistance Training Guide

Exercises and Instructions for Safe Strength

Contact:
Cam McDonald
Ph: 0411380566
E: UQbreastcancerstudy@gmail.com

Safety when resistance training

Serious injury – call the hospital or your medical practitioner immediately

Minor Injury – Please report the injury to study staff ASAP so we can arrange optimal treatment for you – and allow you to continue other exercises.

General Safety

1. Don't perform prolonged stretches before resistance training Only dynamic stretches and light exercises should be performed before training.

2. Always perform your limb mobilising movements before and after any exercise

3. Core strength – before any exercise, think: CORE MUSCLES ON

4. Always continue breathing through the exercise

- **Breath OUT** when you are overcoming the resistance;
- **Breath IN** during the opposite movement.

5. Technique before power – All exercises should be able to be completed with correct technique before any progression in weight occurs.

- If in doubt about the technique– wait until you are supervised before attempting the movement.

6. Complete all prescribed exercises – they are designed to bring balance to your muscles.

Lymphoedema related safety

1. If you have a lymphoedema garment – WEAR IT at all times during warm up, exercise and cool down.

2. Always warm down – stretching and limb movements

3. Do not remove garment until completely cooled down

4. FLARE UP - Stop upper body training and alert study staff, oncologist and/or breast care nurse.

YOUR CORE EXERCISES

Every time you lift!

To perform any exercise properly, there must be a strong foundation from which you are working. Your core muscles protect your lower back, spine, shoulders and neck. If they are not turned on when you lift, then you have a much higher chance of sustaining an injury which will delay your training.

Using the core muscles when you perform your daily activities is essential for it to become a habit.

The 3 essential exercises

The Guidance	The Picture
1. Lie on the ground, knees bent 2. Place one hand on your lower stomach & one hand in the small of your back **3. While breathing out** **i) Turn on your 1's, 2's & 3's** **ii) Draw your belly button to your spine while maintaining the position of your lower back.** 4. Breath normally & hold for 8 seconds	**Abdominal activation**  This is called 'Neutral Spine position' not pushed forward or back, but held firm in the middle
1. Perform lying down or sitting up 2. Turn on your trunk muscles **3. During normal breathing** **i) Roll your shoulders up, back & down** **ii) Try to put your shoulder blades into your back pockets** **iii) Ensure your shoulders are down and not hitched up** 4. Breath normally & hold for 8 seconds	**Scapula setting** 
1. Perform lying or seated 2. Turn on trunk & shoulder muscles **3. While breathing through your nose** **i) Place the tongue on the roof of your mouth** **ii) Maintaining head position, gently nod your chin down and feel a small pressure build at the front of your neck.** 4. Breath normally & hold for 8 seconds	**Deep neck muscles** 

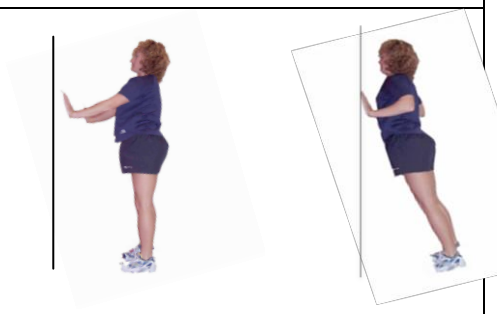
Chest Strength Exercises

Breathing – Always BREATH OUT when pushing the surface or GymStick away from your body

Level I

Wall Push Up

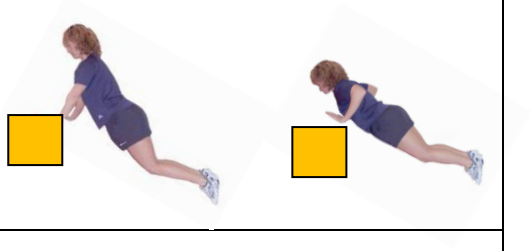
1. Stand at arms length away from the wall
2. Place your hands on the wall below the level of your shoulders, shoulder width apart – fingers pointing up
3. Turn your CORE muscles on
4. Allow your arms to bend taking the strain in your chest muscles.
5. Maintain a straight body from shoulders to toes
6. Using your chest muscles, push back to the starting position



Level II

Kneeling - Box push up

1. On your knees, place your hands the surface shoulder width apart.
2. Core muscles on
3. Lower yourself down by bending your arms
4. Maintain a straight body from shoulders to knees



Level III

Kneeling Push Up

1. On your knees, place your hands the surface shoulder width apart.
2. Core muscles on
3. Lower yourself down by bending your arms
4. Maintain a straight body from shoulders to knees

GymStick Chest Press

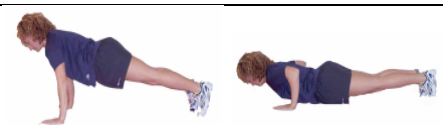
Link the Gymstick straps around each foot & assume a kneeling position.

1. Ensure you have all core muscles turned on
2. Maintain a steady trunk and head throughout movement.
3. Hold the Gymstick with hands outside the width of your shoulders.
4. Push the Gymstick up and away from your body.
5. Control the Gymstick while allowing it come back to the starting position.

LEVEL IV

Full Push-Up

1. Assume a position on your hands and toes
2. Follow instructions similar to push ups on knees.
3. CORE ON – straight & firm body from shoulders to toes
4. DO NOT allow your shoulders to sink into the push up



Back Exercises

Level I

Wall Angel

1. Stand against wall with all core groups turned on.
2. Place slight bend in knees
3. Push your arms back against the wall and you raise them above your head
4. Focus on pushing your arms back while your trunk and head do not move.
5. Drag your arms down while you breathe out.

Level II

Seated Row

1. Place your feet in the long straps of the Gymstick
2. Assume a seated position with knees slightly bent and your back is upright and straight.
3. Turn core muscles on.
4. From a position of outstretched arms, keeping your upper body still, pull the Gymstick towards you.
5. Pull it in at the level just below your chest with arms by your side.

Bent Over Row- Warning (if core is not on your back will be exposed to injury)

1. Straps around your feet. Knees slightly bent, and bending forward at the waist.
2. Turn core muscles on
3. Pull the Gymstick up in a straight line towards your stomach – keep your arms by your side.
4. Control your arms on the way down

Level III

Upright Row

1. Standing with knees slightly bent, straps around your feet
2. Core muscles on
3. Start with your arms relaxed in front of you, holding the stick shoulder width apart.
4. Maintaining your shoulder blade position, pull the bar straight up to the level of your collarbone.
5. Control back down to start position

One armed bent over row

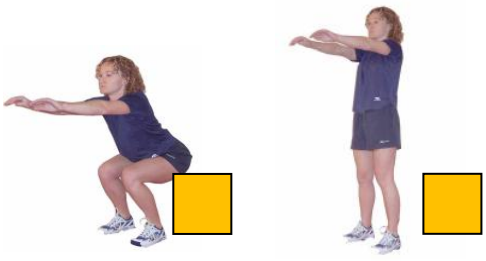
1. Strap bar to both feet and assume a forward lunge position.
2. Use your non-lifting arm as support on your front knee
3. Turn on core muscles
4. Start with your hand in the middle of the Gymstick, arm relaxed by your side.
5. Pull the Gymstick straight up, keeping your arm next to your body.
6. Control the arm back down to the starting position

Lower Body Exercises

Level I

Ia) Sit to Stand

1. Start in seated position in a chair.
2. Turn core muscles on.
3. Arms out in front (or you can use the arm supports)
4. Stand up from the chair as if you are being pulled from the middle of the chest.
5. Push your hips forward to engage the glut muscles.
6. When lowering yourself – push your hips back first and control the movement with your glut muscles.
7. Maintain core activation throughout.



Ib) Calf Raises (with or without GymStick)

1. In a standing position, ensure you have a supporting wall nearby in for safety and balance.
2. Standing up straight with core muscles on
3. Lift up onto your toes and hold for 1 second.
4. Control the movement as you lower yourself down

If using the Gymstick

5. Place it across your shoulders and the back of your neck – DO NOT stick your neck forward.

Ic) Double Glut Bridge

1. Lie on the ground with feet shoulder width apart.
2. Squeeze your gluts together tightly
3. Use your gluts to push your hips up.
4. Slowly lower your hips, but don't let them touch the ground between repetitions.
5. Stop when you feel like your hamstrings (the muscles on the back of the thigh are being used)

TIP: Put your fingers on your glut muscles – it will help you turn them on.

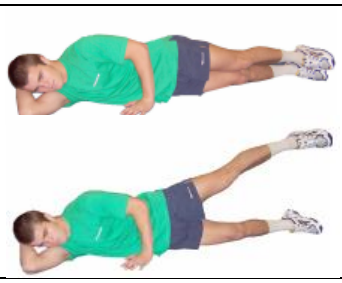
LEVEL II

IIa) Squats – Body Weight

1. Feet shoulder width apart, standing up (can do it in front of a chair/box)
2. Core muscles on
3. Push your hips back - keeping your head upright and shoulders back (as if you are sitting down)
4. Go down as far as you feel comfortable for balance
5. When coming up – push hips forward and drive from your glut muscles.

IIb) Lunge – Body Weight

1. Take a normal to large step forward
2. Turn on core muscles
3. Make sure your feet are pointing straight ahead
4. Lift the heel of the hind leg
5. Lower the back knee toward the ground until the front thigh is parallel to the ground – keep your head up and shoulders back
6. Use your leading leg's glut muscles to push you up

Leg Exercises - LEVEL II cont'd	
IIc) Leg Abduction – Lying 1. Lie on your side with your legs in line with your upper body and knees slightly bent behind you 2. Roll and push hips slightly forward 3. Slowly lift and lower the top leg. 4. Toes face straight ahead	
IId) Single Leg Calf Raise As above with double leg – standing on one leg, perform equal repetitions on both sides (start exercise without Gymstick)	
LEVEL III	
GymStick Squat 1. As per normal squat (Exercise IIa) 2. Core muscles on 3. Rest Gymstick on shoulders behind the neck. 4. Keep head up and shoulders back throughout	
Single Leg Glut Bridge 1. Set up as per double leg glut bridge (Exercise Ic) 2. Squeeze glut muscles together 3. Push hips up – keeping the hips even and horizontal to the ground. 4. Stop when the hamstring muscles are felt throughout the movement.	
Dead Lift – Gymstick 1. Strap feet into Gymstick 2. Core muscles on – **maintain neutral spine throughout** 3. Hands shoulder width apart 3. Start in standing position 4. Push hips back - keeping arms straight at all times. 5. Bend forward with control keeping the bar close to the thighs and shins. 6. Return to standing by pushing hips forward	
Leg abduction – Gymstick 1. Put the lower Gymstick loop on the moving leg. 2. Place Gymstick on the opposite side – 40-60cm away from the stationary foot. 3. Maintaining stable trunk and upper body – lift foot away from the body. 4. Control the movement back down to the starting position 5. DO NOT – sink into the stationary hip (see Cam for more instructions)	

Abdominal and Trunk Exercises

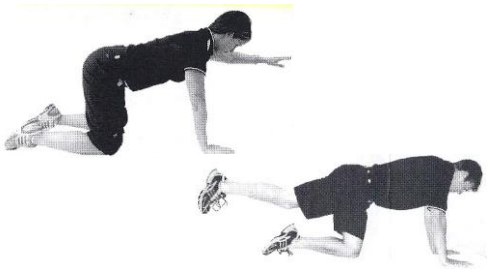
Always place your tongue at the roof of your mouth when performing abdominal exercises

LEVEL I

4 Point Exercise

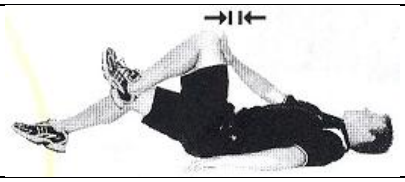
1. Start on all 4's. Activate core muscles in neutral spine position.
2. Keeping hips and shoulder steady – raise one arm and hold for 8s, relax for 15s, then repeat
3. Repeat for each arm and leg individually

TIP: Use a tennis ball on the back to ensure stability throughout movement.



Abdominal Obliques

1. In lying position, press opposite hand and knee together.
2. Do not strain your neck
3. Hold for 8s, relax for 15s and repeat



Level II

Single Leg Raises

1. Turn on trunk muscles
2. Slowly lift one leg off the ground
3. Maintain neutral spine at all times
4. Continue breathing normally throughout

Lateral Trunk Raises

1. Strap GymStick to feet and place on shoulders at the back of your neck.
2. Turn on core muscles
3. Push hips forward to maintain straight posture
4. Bend from side to side – ensure it is only lateral movement
5. Maintain slow and controlled movements throughout

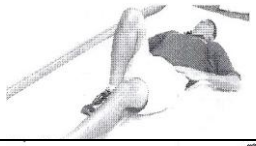
4 point Exercise (II)

1. Start in same position as per Level I.
2. Maintaining level hips and shoulders – extend opposite arm and leg and hold for 8 seconds, rest for 15s then repeat with the other arm and leg.
3. Place a tennis ball or broomstick to test stability.

LEVEL III

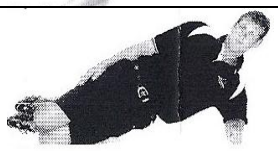
Single leg lift – straight leg

1. As single leg lift above – but straightening the leg
2. Extend leg and hold just above the floor.
3. Maintain neutral spine position – 8s on, 15s rest
4. If you feel it in your lower back, STOP & back off.



Side Bridge – Knees

1. Lie on side, rest on your forearm directly below shoulder.
2. Lift your hips & push them forward slightly – hold 10s
3. Maintain strong shoulders throughout



Arms and Shoulder Exercises

LEVEL I

Bicep Curl

1. Stand with feet and hands shoulder width apart.
2. Slightly bent knees and core muscles on
3. Start with arms straight down holding the bar.
4. Keeping your shoulders still, fully bend your elbows.
5. Control the movement back down
6. Maintain steady frame at all times.

Front Raise - Lying

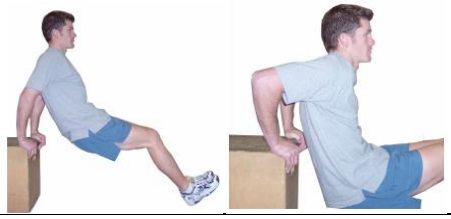
1. Strap Gymstick to your feet in lying position, knees slightly bent.
2. Stabilise shoulders and core
3. Keeping straight arms, lift the bar up to be in line with your chin.
4. Slowly lower the bar back down

LEVEL II

Dips – On Chair/Box

1. Hands on bench (fingers facing forward or back)
2. Support body weight partially with feet on ground
3. Lower body until arms are parallel to the ground.
4. Ensure your back is as close to the bench as possible

TIP: The closer your feet are to your body, the easier it is



LEVEL III

Shoulder Press – Standing

1. Strap to feet and grip with hands outside shoulder width.
2. Slightly bend knees, turn on core muscles
3. Start with bar across the your collar bone.
4. Lift the bar up and just in front of your face.
5. DO NOT lean back into this exercise. Maintain strong core muscles throughout movement.

TIP: Easier to control when seated.

If you feel any discomfort in your lower back, you are either:
1) leaning back too far – bend your knees more;
2) Not turning your core muscles on – activate them before each lift.

Triceps Extension

1. Set up the same as above exercise.
2. Start position as shown – bar at the back of your head
3. Elbows facing straight ahead.
4. Straighten your elbow until bar is directly above your head.
5. Always maintain the position of your upper arm. Only allow the forearm to move.

-

Stretching

Record Sheet – Aerobic & Resistance training

Exercise/ Date	*Example*									
Warm up	*Walking + Stretches*									
	Load	*Reps per set*	Load	Reps per set	Load	Reps per set	Load	Reps per set	Load	Reps per set
Chest Press	*Wind x 2*	*1 x 12* *1 x 10*								
Squats	*BW*	*1 x 15* *1 x 10*								
Seated Row	*Wind x 1*	*1 x 8* *1 x 8*								
Walking	***3.3km*** ***25 min***	***11-12 RPE*** ***120bpm***								

Stretches completed:

1 2 3 4 5 6 7 8

Record Sheet – Walking or aerobic training

Pain/Aches: Shoulders Neck Lower back Hips Knees Ankles

Exercise/ Date										
Warm up										
Cool down Exercises performed										

Comments:

Appendix 5 – Literature Review Documents

A-5.1 Search terms for literature review

Subject	Search terms
Breast cancer survivors	breast neoplasm, cancer of the breast, breast cancer survivor, breast neoplasm risk
Cancer	Neoplasm, cancer survivor, tumour, cancer cachexia, cancer wasting
Body composition	Adiposity, cachexia, body composition, body constitution, body fat distribution, body mass index, body size, body weight, body weights and measures, body weight changes, lean body mass, muscles, muscle skeletal, muscular atrophy, muscle weakness, sarcopenia, obesity, overweight, skin fold thickness, thinness, waist-hip ratio, weight gain, weight loss
Exercise intervention	Aerobic exercise, resistance exercise, physical fitness
Dietary interventions	Carbohydrates, lipids, fats, dietary fats, plant oils, dietary protein, macronutrient, Energy intake, Food preferences, Energy metabolism, Minerals, Diet therapy
Omega-3 fatty acids	Omega 3 fatty acids; fats, unsaturated; fatty acids; decanoic acids; eicosanoic acids; fatty acids, unsaturated; oils; fish oils

Table A5.1 Literature search subject and corresponding key words

The search parameters contained the following limits: human trials, published in English, >18yrs of age, controlled trials.
The following subjects were combined with breast cancer survivors & body composition: exercise intervention; dietary interventions; omega-3 fatty acids.
The following subjects were combined with omega-3 fatty acids: body composition; cancer; exercise intervention; dietary interventions.

A-5.2 Literature review quality assessment documents

A-5.2.1 NHMRC Evidence Hierarchy Document

Table 1 NHMRC Evidence Hierarchy: designations of 'levels of evidence' according to type of research question (including explanatory notes)

Level	Intervention [1]	Diagnostic accuracy [2]	Prognosis	Aetiology [3]	Screening Intervention
I [4]	A systematic review of level II studies	A systematic review of level II studies	A systematic review of level II studies	A systematic review of level II studies	A systematic review of level II studies
II	A randomised controlled trial	A study of test accuracy with: an independent, blinded comparison with a valid reference standard,[5] among consecutive persons with a defined clinical presentation[6]	A prospective cohort study[7]	A prospective cohort study	A randomised controlled trial
III-1	A pseudorandomised controlled trial (i.e. alternate allocation or some other method)	A study of test accuracy with: an independent, blinded comparison with a valid reference standard,[5] among non-consecutive persons with a defined clinical presentation[6]	All or none[8]	All or none[8]	A pseudorandomised controlled trial (i.e. alternate allocation or some other method)
III-2	A comparative study with concurrent controls: ▪ Non-randomised, experimental trial[9] ▪ Cohort study ▪ Case-control study ▪ Interrupted time series with a control group	A comparison with reference standard that does not meet the criteria required for Level II and III-1 evidence	Analysis of prognostic factors amongst persons in a single arm of a randomised controlled trial	A retrospective cohort study	A comparative study with concurrent controls: ▪ Non-randomised, experimental trial ▪ Cohort study ▪ Case-control study
III-3	A comparative study without concurrent controls: ▪ Historical control study ▪ Two or more single arm study[10] ▪ Interrupted time series without a parallel control group	Diagnostic case-control study[6]	A retrospective cohort study	A case-control study	A comparative study without concurrent controls: ▪ Historical control study ▪ Two or more single arm study
IV	Case series with either post-test or pre-test/post-test outcomes	Study of diagnostic yield (no reference standard)[11]	Case series, or cohort study of persons at different stages of disease	A cross-sectional study or case series	Case series

6

Source: NHMRC additional levels of evidence and grades for recommendations for developers of guidelines. PILOT PROGRAM 2005 – 2007. National Health and Medical Research Council http://www.nhmrc.gov.au/_files_nhmrc/file/guidelines/levels_grades05.pdf

A-5.2.2 ADA Article Quality Criteria Checklist

Appendix 8: Quality Criteria Checklist: Primary Research

Symbols Used

\+ **Positive:** Indicates that the report has clearly addressed issues of inclusion/exclusion, bias, generalizability, and data collection and analysis.

-- **Negative:** Indicates that these issues have not been adequately addressed.

∅ **Neutral:** Indicates that the report is neither exceptionally strong nor exceptionally weak.

Quality Criteria Checklist: Primary Research

RELEVANCE QUESTIONS	
5. Would implementing the studied intervention or procedure (if found successful) result in improved outcomes for the patients/clients/population group? (NA for some Epi studies)	Yes No Unclear N/A
6. Did the authors study an outcome (dependent variable) or topic that the patients/clients/population group would care about?	Yes No Unclear N/A
7. Is the focus of the intervention or procedure (independent variable) or topic of study a common issue of concern to dietetics practice?	Yes No Unclear N/A
8. Is the intervention or procedure feasible? (NA for some epidemiological studies)	Yes No Unclear N/A
If the answers to all of the above relevance questions are "Yes," the report is eligible for designation with a plus (+) on the Evidence Quality Worksheet, depending on answers to the following validity questions.	
VALIDITY QUESTIONS	
11. Was the research question clearly stated? 1.1 Was the specific intervention(s) or procedure (independent variable(s)) identified? 1.2 Was the outcome(s) (dependent variable(s)) clearly indicated? 1.3 Were the target population and setting specified?	Yes No Unclear N/A
12. Was the selection of study subjects/patients free from bias? 2.1 Were inclusion/exclusion criteria specified (e.g., risk, point in disease progression, diagnostic or prognosis criteria), and with sufficient detail and without omitting criteria critical to the study? 2.2 Were criteria applied equally to all study groups? 2.3 Were health, demographics, and other characteristics of subjects described? 2.4 Were the subjects/patients a representative sample of the relevant population?	Yes No Unclear N/A
13. Were study groups comparable? 3.1 Was the method of assigning subjects/patients to groups described and unbiased? (Method of randomization identified if RCT) 3.2 Were distribution of disease status, prognostic factors, and other factors (e.g., demographics) similar across study groups at baseline? 3.3 Were concurrent controls used? (Concurrent preferred over historical controls.) 3.4 If cohort study or cross-sectional study, were groups comparable on important confounding factors and/or were preexisting differences accounted for by using appropriate adjustments in statistical analysis? 3.5 If case control study, were potential confounding factors comparable for cases and controls? (If case series or trial with subjects serving as own control, this criterion is not applicable. Criterion may not be applicable in some cross-sectional studies.) 3.6 If diagnostic test, was there an independent blind comparison with an appropriate reference standard (e.g., "gold standard")?	Yes No Unclear N/A
14. Was method of handling withdrawals described? 4.1 Were follow up methods described and the same for all groups? 4.2 Was the number, characteristics of withdrawals (i.e., dropouts, lost to follow up, attrition rate) and/or response rate (cross-sectional studies) described for each group? (Follow up goal for a strong study is 80%.) 4.3 Were all enrolled subjects/patients (in the original sample) accounted for? 4.4 Were reasons for withdrawals similar across groups?	Yes No Unclear N/A

	4.5 If diagnostic test, was decision to perform reference test not dependent on results of test under study?	
15.	**Was blinding used to prevent introduction of bias?**	Yes No Unclear N/A
	5.1 In intervention study, were subjects, clinicians/practitioners, and investigators blinded to treatment group, as appropriate?	
	5.2 Were data collectors blinded for outcomes assessment? (If outcome is measured using an objective test, such as a lab value, this criterion is assumed to be met.)	
	5.3 In cohort study or cross-sectional study, were measurements of outcomes and risk factors blinded?	
	5.4 In case control study, was case definition explicit and case ascertainment not influenced by exposure status?	
	5.5 In diagnostic study, were test results blinded to patient history and other test results?	
16.	**Were intervention/therapeutic regimens/exposure factor or procedure and any comparison(s) described in detail? Were intervening factors described?**	Yes No Unclear N/A
	6.1 In RCT or other intervention trial, were protocols described for all regimens studied?	
	6.2 n observational study, were interventions, study settings, and clinicians/provider described?	
	6.3 Was the intensity and duration of the intervention or exposure factor sufficient to produce a meaningful effect?	
	6.4 Was the amount of exposure and, if relevant, subject/patient compliance measured?	
	6.5 Were co-interventions (e.g., ancillary treatments, other therapies) described?	
	6.6 Were extra or unplanned treatments described?	
	6.7 Was the information for 6.4, 6.5, and 6.6 assessed the same way for all groups?	
	6.8 In diagnostic study, were details of test administration and replication sufficient?	
17.	**Were outcomes clearly defined and the measurements valid and reliable?**	Yes No Unclear N/A
	7.1 Were primary and secondary endpoints described and relevant to the question?	
	7.2 Were nutrition measures appropriate to question and outcomes of concern?	
	7.3 Was the period of follow-up long enough for important outcome(s) to occur?	
	7.4 Were the observations and measurements based on standard, valid, and reliable data collection instruments/tests/procedures?	
	7.5 Was the measurement of effect at an appropriate level of precision?	
	7.6 Were other factors accounted for (measured) that could affect outcomes?	
	7.7 Were the measurements conducted consistently across groups?	
18.	**Was the statistical analysis appropriate for the study design and type of outcome indicators?**	Yes No Unclear N/A
	8.1 Were statistical analyses adequately described the results reported appropriately?	
	8.2 Were correct statistical tests used and assumptions of test not violated?	
	8.3 Were statistics reported with levels of significance and/or confidence intervals?	
	8.4 Was "intent to treat" analysis of outcomes done (and as appropriate, was there an analysis of outcomes for those maximally exposed or a dose-response analysis)?	
	8.5 Were adequate adjustments made for effects of confounding factors that might have affected the outcomes (e.g., multivariate analyses)?	
	8.6 Was clinical significance as well as statistical significance reported?	
	8.7 If negative findings, was a power calculation reported to address type 2 error?	
19.	**Are conclusions supported by results with biases and limitations taken into consideration?**	Yes No Unclear N/A
	9.1 Is there a discussion of findings?	
	9.2 Are biases and study limitations identified and discussed?	
20.	**Is bias due to study's funding or sponsorship unlikely?**	Yes No Unclear N/A
	10.1 Were sources of funding and investigators' affiliations described?	
	10.2 Was there no apparent conflict of interest?	
MINUS/NEGATIVE (-)		
If most (six or more) of the answers to the above validity questions are "No," the report should be designated with a minus (-) symbol on the Evidence Worksheet.		

Appendix 6

A-6.1 Assay Method for Hs-CRP analysis

SIEMENS

CE

ADVIA® Chemistry
Systems

Siemens Healthcare Diagnostics Inc.

Instructions for Use
2008-10

Wide Range C-Reactive Protein (wrCRP)

System	New Information
ADVIA® 1200	Update to Siemens Healthcare Diagnostics
ADVIA 1650/1800	Update to Siemens Healthcare Diagnostics
ADVIA 2400	Update to Siemens Healthcare Diagnostics

NOTE: For an explanation of the symbols in this document and associated products, refer to *Understanding the Symbols* located on the introductory page of the Methods Directory.

Method Summary

Item	Description	
Method Principle	Latex enhanced immunoturbidimetric	
Specimen Type	Human serum and plasma (lithium heparin)	
On-board Stability	ADVIA 1200:	35 days
	ADVIA 1650/1800:	35 days
	ADVIA 2400:	21 days
Reagent Storage Temperature	2–8°C	
Calibration Frequency	ADVIA 1200:	21 days
	ADVIA 1650/1800:	21 days
	ADVIA 2400:	21 days
Reagent Blank (RBL) Frequency	At time of method calibration	
Reaction Type	2-point (EPA)	
Measurement Wavelength	571 nm	
Standardization	IRMM Reference Material CRM 470	

Siemens Healthcare Diagnostics Inc., Tarrytown, NY 10591-5097 USA
04069518, Rev. D, 2008-10

Item	Description	
Analytical Range	**System**	**Serum/Plasma***
	ADVIA 1200	0.002–(15.6–16.4) mg/dL (0.02–[156–164] mg/L)
	ADVIA 1650/1800	0.012–(15.6–16.4) mg/dL (0.12–[156–164] mg/L)
	ADVIA 2400	0.003–(15.6–16.4) mg/dL (0.03–[156–164] mg/L)
	*The wrCRP concentration in the ADVIA Wide Range C-Reactive Protein Calibrator Level 6, varies from 15.6 – 16.4 mg/dL (156 – 164 mg/L).	
Expected Values	Adults:	0–0.5 mg/dL (0–5.0 mg/L)
	Newborns, cord blood:	< 0.06 mg/dL (< 0.6 mg/L)
	Infants, 4 days–1 month:	< 0.16 mg/dL (< 1.6 mg/L)
Reagent Code	74038	
Calibrators	ADVIA Chemistry Wide Range C-Reactive Protein Calibrators: REF 00337402 (PN B03-4815-01)	

Intended Use [1-4]

For *in vitro* diagnostic use in the quantitative determination of the concentration of C-Reactive Protein in human serum and plasma on the ADVIA Chemistry systems. Such measurements are used in the detection and evaluation of infection, tissue injury, inflammatory disorders, and associated diseases. Increases in CRP values are non-specific for many disease processes and should not be interpreted without a complete clinical evaluation.

This method is referred to as wide range CRP (wrCRP) because of the relatively wide analytical range that can be measured.

Summary and Explanation

The wrCRP method measures CRP in serum and plasma by a latex-enhanced immunoturbidimetric assay. It is based on the principle that the analyte concentration is a function of the intensity of scattered light caused by the latex aggregates. The latex particles coated with anti-CRP rapidly agglutinate in the presence of C-Reactive Protein-forming aggregates.

Principles of the Procedure

The wrCRP latex reagent is a suspension of uniform polystyrene latex particles coated with anti-CRP antibody. When serum or plasma containing CRP is mixed with the latex reagent, agglutination takes place resulting in an increase in the turbidity. This turbidity is measured at 571 nm. The CRP concentration in serum or plasma is determined from a calibration curve that is generated with the calibrators.

Reagents

The reagents are packaged as listed below. Components of the package are available as a kit only.

REF (PN) Container Size	Symbol	Contents	Amount	No. of Tests
03108390 (B01-4800-01)	wr CRP	Wide Range C-Reactive Protein Reagents		2 x 220
20-mL	wr CRP R1	Reagent 1	2 x 13.0 mL	
20-mL	wr CRP R2	Reagent 2	2 x 13.0 mL	
00829585	wr CRP	Wide Range C-Reactive Protein Reagents		7 x 315
20-mL	wr CRP R1	Reagent 1	7 x 18 mL	
20-mL	wr CRP R2	Reagent 2	7 x 18 mL	

Components and Concentrations

Reagent	Component	Concentration
Reagent 1	Glycine	170 mmol/L
	Sodium chloride	100 mmol/L
	Sodium EDTA disodium salt dihydrate	50 mmol/L
	Sodium azide	0.09% w/v
Reagent 2	anti–CRP antibody (rabbit) – synthetic latex	Lot specific
	Sodium azide	0.09% w/v

CAUTION! This device contains material of animal origin and should be handled as a potential carrier and transmitter of disease.

NOTE: Sodium azide can react with copper and lead plumbing to form explosive metal azides. If disposal into a drain is in compliance with federal, state, and local requirements, flush reagents with a large amount of water to prevent the buildup of azides.

For *In Vitro* Diagnostic Use.

Reagent Preparation and Use

Reagents are ready to use. Before use, gently swirl the reagent to disrupt bubbles and assure homogeneity. If bubbles still exist or foam is present, using a clean transfer pipette, aspirate them from the reagent container prior to use.

On-board Reagent Stability (OBS)

System	Stability
ADVIA 1200	35 days
ADVIA 1650/1800	35 days
ADVIA 2400	21 days

For all systems, unopened reagents are stable until the expiration date printed on the product label when stored at 2–8°C. Do not freeze reagents.

For additional details, refer to the *Methods Introduction* section of the system-specific Operator's Guide.

Sample Handling

Siemens Healthcare Diagnostics recommends using serum and plasma (lithium heparin) for this method.

For additional details, refer to Sample Collection and Preparation in the *Methods Introduction* section of the system-specific Operator's Guide.

For instructions on how to load reagents and run samples, refer to the *Daily Operations* section of the system-specific Operator's Guide.

Materials Required but not Provided

The following list contains the materials required, but not provided, to perform this method:

- sample containers
- system solutions
- calibrator (refer to the *Method Summary* section for the REFs)
- control materials
- reagent container adapters:
  - 20-mL adapter (REF 02404085; PN 094-0159-01) for 40-mL slot (ADVIA 1200/1800)
  - 20-mL adapter (REF 05249323; PN 073-0936-01) for 70-mL slot (ADVIA 1800)
  - 20-mL adapter (REF 00771668; PN 073-0345-02) for 70-mL slot (ADVIA 1650/2400)

For storage and stability information, refer to the package insert.

Calibration

Refer to the package insert supplied with the ADVIA Chemistry Wide Range C-Reactive Protein Calibrators (REF 00337402; PN B03-4815-01) for handling instructions and values. For setup and use instructions, refer to the *Calibration Overview* section of the system-specific Operator's Guide.

Calibration Frequency

Perform a calibration when this method is implemented on the system. You must recalibrate after the following events:

- when the reagent lot number changes
- after replacing critical optical or hydraulic components
- when indicated by quality control procedures

Siemens has validated the calibration stability for this method as shown in the following table:

System	Minimum Calibration Stability*
ADVIA 1200	21 days
ADVIA 1650/1800	21 days
ADVIA 2400	21 days

*or whenever indicated by quality control data

Siemens recommends calibrating new reagent packs if the previous reagent pack was calibrated any time during its on-board stability, other than as a fresh pack.

Individual laboratory quality control programs and procedures may require more frequent calibration.

Reagent Blank (RBL) Frequency

The RBL is measured at the time of method calibration.

Quality Control

Siemens recommends the use of quality control material from Bio-Rad Laboratories with at least 2 levels (low and high). A satisfactory level of performance is achieved when the analyte values obtained are within the Acceptable Control Range for the system or within your range, as determined by an appropriate internal laboratory quality control scheme.

The actual frequency of control in a laboratory is based on many factors, such as workflow, system experience, and government regulation. Each laboratory should evaluate the controls based on the frequency established by their laboratory guidelines. When the method is performed, analyze at least 2 levels of controls daily.

Also, assay controls under the following conditions:

- whenever you use a new reagent lot
- following the performance of any system maintenance, cleaning, or troubleshooting procedure
- after performing a new calibration

For more information, refer to the *Quality Control Overview* section of the system-specific Operator's Guide.

Limitations of the Procedure [5]

A number of substances cause physiological changes in serum or plasma analyte concentrations. A comprehensive discussion of possible interfering substances, their serum or plasma concentrations, and their possible physiological involvements is beyond the scope of this document. Consult listed reference for specific details on known potential interfering substances.[5]

As with any chemical reaction, you must be alert to the possible effect on results of unknown interferences from medications or endogenous substances. The laboratory and physician must evaluate all patient results in light of the total clinical status of the patient.

Interferences

Siemens tested the following potential interferents and found the results shown below:

ADVIA 1200

Interferent	Interferent Level	wrCRP Sample Concentration	Interference*
Bilirubin (conjugated and unconjugated)	50 mg/dL (855 µmol/L)	1 mg/dL (10 mg/L)	NSI
Hemolysis (hemoglobin)	1000 mg/dL (10.0 g/L)	1 mg/dL (10 mg/L)	NSI
Lipemia (from Intralipid)	750 mg/dL (8.48 mmol/L)**	1 mg/dL (10 mg/L)	NSI
Lipemia (from triglycerides concentrate)	1000 mg/dL (11.30 mmol/L)**	1 mg/dL (10 mg/L)	NSI

*NSI = No Significant Interference. A percentage effect ≥10% is considered a significant interference.

**as triolein

ADVIA 1650/1800

Interferent	Interferent Level	wrCRP Sample Concentration	Interference*
Bilirubin (conjugated/unconjugated)	30 mg/dL (513 µmol/L)	0.068 mg/dL (0.68 mg/L)	NSI
Hemolysis (hemoglobin)	694 mg/dL (6.94 g/L)	0.068 mg/dL (0.68 mg/L)	NSI
Lipemia (from Intralipid)	500 mg/dL (5.65 mmol/L)**	0.068 mg/dL (0.68 mg/L)	NSI
	1000 mg/dL (11.30 mmol/L)**	0.458 mg/dL (4.58 mg/L)	NSI

*NSI = No Significant Interference. A percentage effect ≥10% is considered a significant interference.

**as triolein

ADVIA 2400

Interferent	Interferent Level	wrCRP Sample Concentration	Interference*
Bilirubin (conjugated/unconjugated)	25 mg/dL (428 µmol/L)	0.094 mg/dL (0.94 mg/L)	NSI
Hemolysis (hemoglobin)	1000 mg/dL (10.0 g/L)	0.102 mg/dL (1.02 mg/L)	NSI
Lipemia (from Intralipid)	800 mg/dL (9.04 mmol/L)**	0.094 mg/dL (0.94 mg/L)	NSI

*NSI = No Significant Interference. A percentage effect ≥10% is considered a significant interference.

**as triolein

Performance Characteristics

Precision [6]

Each sample was assayed 2 times per run, 2 runs per day, for at least 10 days. Precision estimates were computed according to CLSI document EP05-A2, *Evaluation of Precision Performance of Quantitative Measurement Methods*; Approved Guideline.[6]

Data contained in this section represents typical performance for ADVIA Chemistry systems. Your laboratory data may differ from these values.

Conversion factor: mg/dL x 10 = mg/L

ADVIA 1200

		Within-Run		Total	
Specimen Type	**Level**	**SD**	**CV (%)**	**SD**	**CV (%)**
		Common Units (mg/dL)			
wrCRP Control 1	0.110	0.004	3.7	0.006	5.1
wrCRP Control 2	0.409	0.009	2.3	0.013	3.1
Control 1	2.636	0.022	0.8	0.030	1.1
Control 2	5.161	0.069	1.3	0.089	1.7
Control 3	8.030	0.171	2.1	0.183	2.3
		SI Units (mg/L)			
wrCRP Control 1	1.10	0.04	3.7	0.06	5.1
wrCRP Control 2	4.09	0.09	2.3	0.13	3.1
Control 1	26.36	0.22	0.8	0.30	1.1
Control 2	51.61	0.69	1.3	0.89	1.7
Control 3	80.30	1.71	2.1	1.83	2.3

ADVIA 1650/1800

		Within-Run		Total	
Specimen Type	**Level**	**SD**	**CV (%)**	**SD**	**CV (%)**
		Common Units (mg/dL)			
wrCRP Control 1	0.079	0.003	3.3	0.003	3.5
wrCRP Control 2	0.406	0.007	1.8	0.008	1.9
		SI Units (mg/L)			
wrCRP Control 1	0.79	0.03	3.3	0.03	3.5
wrCRP Control 2	4.06	0.07	1.8	0.08	1.9

ADVIA 2400

		Within-Run		Total	
Specimen Type	**Level**	**SD**	**CV (%)**	**SD**	**CV (%)**
		Common Units (mg/dL)			
wrCRP Control 1	0.225	0.007	3.2	0.011	4.9
wrCRP Control 2	1.035	0.017	1.7	0.022	2.1
Control 1	4.996	0.257	5.2	0.390	7.8
		SI Units (mg/L)			
wrCRP Control 1	2.25	0.07	3.2	0.11	4.9
wrCRP Control 2	10.35	0.17	1.7	0.22	2.1
Control 1	49.96	2.57	5.2	3.90	7.8

Analytical Range

This method measures the wrCRP concentration in serum and plasma ranging from the minimum detectable concentration (MDC) to the wrCRP concentration in the highest level of the calibrator according to the table shown below. MDC is an estimation based on two times the within-run standard deviation of the zero calibrator.

System	Serum/Plasma*
ADVIA 1200	0.002–(15.6–16.4) mg/dL (0.02–[156–164] mg/L)
ADVIA 1650/1800	0.012–(15.6–16.4) mg/dL (0.12–[156–164] mg/L)
ADVIA 2400	0.003–(15.6–16.4) mg/dL (0.03–[156–164] mg/L)

*The wrCRP concentration in the ADVIA Wide Range C-Reactive Protein Calibrator Level 6 varies from 15.6 – 16.4 mg/dL (156 – 164 mg/L).

Siemens has validated an automatic rerun condition for this method that extends the reportable ranges for serum and plasma to:

- 78.0 mg/dL (780 mg/L) for ADVIA 1200
- 62.4 mg/dL (624 mg/L) for ADVIA 1650, 1800, and 2400

Prozone Effect

A prozone effect has been observed to occur at concentrations greater than 95 mg/dL (950 mg/L).

Expected Values [3,4]

The following table lists the reference ranges for this method:

Sample Type	Reference Range
Adults	0–0.5 mg/dL (0–5.0 mg/L)
Newborns, cord blood	<0.06 mg/dL (<0.6 mg/L)
Infants from 4th day of life to 1 month	<0.16 mg/dL (<1.6 mg/L)

Siemens provides this information for reference. Each laboratory should establish its own normal range. You can enter normal range values and abnormal range values at the Analytical Parameters (Chemistry) window.

System Correlation

The performance of the applicable method (y) was compared with the performance of the same method on the comparison system (x).

ADVIA 1200

Specimen Type	Comparison System (x)	N	Regression Equation	Sy.x	r	Sample Range
Serum	ADVIA 1650	98	y = 0.99x - 0.047 y = 0.99x - 0.47	0.129 1.29	0.996 0.996	0.99–5.99 mg/dL 9.9–59.9 mg/L
Plasma*	ADVIA 1200 (serum)	49	y = 1.00x + 0.002 y = 1.00x + 0.02	0.075 0.75	0.998 0.998	0.62–4.75 mg/dL 6.2–47.5 mg/L

*lithium heparin

ADVIA 1650/1800

Specimen Type	Comparison System (x)	N	Regression Equation	Sy.x	r	Sample Range
Serum	Hitachi CRP	60	y = 0.96x - 0.037 y = 0.96x - 0.37	0.135 1.35	0.999 0.999	0–15.81 mg/dL 0–158.1 mg/L
Plasma*	ADVIA 1650 (serum)	65	y = 0.99x - 0.004 y = 0.99x - 0.04	0.110 1.10	0.998 0.998	0.02–6.98 mg/dL 0.20–69.8 mg/L

*lithium heparin

ADVIA 2400

Specimen Type	Comparison System (x)	N	Regression Equation	Sy.x	r	Sample Range
Serum	ADVIA 1650	94	y = 1.03x + 0.023 y = 1.03x + 0.23	0.288 2.88	0.998 0.998	0.03–15.11 mg/dL 0.30–151.1 mg/L
Plasma*	ADVIA 2400 (serum)	48	y = 1.00x - 0.00 y = 1.00x - 0.00	0.080 0.80	0.999 0.999	0.04–14.30 mg/dL 0.40–143.0 mg/L

*lithium heparin

Standardization

The ADVIA wrCRP method is traceable to the IRMM reference material CRM 470 from IFCC (International Federation of Clinical Chemistry). Recovery averaged 105% for the ADVIA 1200, 95% for the ADVIA 1650/1800, and 99% for the ADVIA 2400, of the target concentration. Assigned values of ADVIA Chemistry Wide Range C-Reactive Protein Calibrators are traceable to this standardization.

References

1. Ng PC. *Archives of Disease in Childhood.* 1997; 77(3):221-227.
2. Claus. J*ournal of Laboratory and Clinical Medicine.* 1976; 87(1): 20-127.
3. Konsensuswerte der DeutschenGesellschaft fur Laboratoriums-medizin, der DeutschenGesellschaft fur Klinische Chemie und des Verbandes der Diagnostica-Industrie e. V. (VDGH). *Clin Lab.* 1995; 41:743-748.
4. Kindmark CO. The concentration of C-reactive protein in sera from healthy individuals. *Scand J Clin Lab Invest.* 1972; 229:407-111.
5. Young DS. *Effects of Drugs on Clinical Laboratory Tests*. 3rd ed. Washington: AACC Press; 1990.
6. Clinical and Laboratory Standards Institute (formerly NCCLS). *Evaluation of Precision Performance of Quantitative Measurement Methods; Approved Guideline - Second Edition.* Wayne, PA: Clinical and Laboratory Standards Institute; 2004. NCCLS Document EP05-A2.

Technical Assistance

For customer support, please contact your local technical support provider or distributor.

www.siemens.com/diagnostics

Trademarks

Origin: US

Siemens Healthcare Diagnostics Inc.
Tarrytown, NY 10591-5097 USA

EC REP Siemens Healthcare Diagnostics Ltd
Sir William Siemens Sq.
Frimley, Camberley, UK GU16 8QD

Siemens Healthcare
Diagnostics Pty Ltd
885 Mountain Highway
Bayswater Victoria 3153
Australia

A-6.2 Assay method for erythrocyte fatty acids

Determination Of Essential Fatty Acids In Serum, Plasma Or Red Cells

CLINICAL SIGNIFICANCE:

Essential fatty acids (EFAs) cannot be produced by the body but must be included as an essential dietary factor. EFAs are constituents of all membranes in all tissues of the body. They play a vital role in determining the biological properties of these membranes and therefore EFA deficiency can lead to profound disturbances in all tissues. EFAs are precursors of highly reactive, short-lived molecules, the prostaglandins and leucotrienes. Chronic deficiency of EFAs is potentially implicated in coronary disease, diabetes mellitus, breast and menstrual disorders, immunity and inflammation and psychiatric disorder.

PRINCIPLE:

The sample of serum is extracted with a mixture of chloroform and methanol. The chloroform fraction is then dried under nitrogen and the lipid fraction is trans-esterified to methylesters by reconstitution in Meth. Prep. II reagent. This methylated extract is then analysed by Gas Liquid Chromatography using flame ionisation detection (GLC-FID).

SPECIMEN:

Plasma/Serum: 1 mL Lithium Heparin/EDTA, frozen, fasting
Red cells: 5 mL Lithium Heparin/EDTA whole blood, not frozen, received within 2 days of collection, fasting.

REAGENTS:

Reagent	Catalogue no	Supplier	Storage
Methanol, AR	Any brand	Any brand	Ambient
Chloroform, AR	Any brand	Any brand	Ambient
Potassium Chloride (KCL)	Any brand	Any brand	Ambient
Meth Prep II	18007	Alltech	Ambient
FAME-Mix C4-C24	18919	Supelco	-20°C
Sodium sulphate, granular	Any brand	Any brand	Ambient
Silanized glass wool	Any brand	Any brand	Ambient

PREPARATION:

- **Methanol/Chloroform Mixture (1:2 v/v):** Stable at room temperature for 12 months.
- **0.1 M Potassium Chloride (KCL):** Dissolve 1.86 g KCL in 250 mL of distilled water. Stable at room temperature for 6 months. ,
- **Qualitative Standards:** Fatty Acids Methyl Esteos Mix C4 ÷C24, catalogue No 18919 Lot No 18572 from Supelco.
- **Sodium sulphate:** Before use has to be dried in the muffle furnace at 400 °C for 2 hours.
- **Internal quality control:** A pooled serum control from normal patient sera is used as an internal quality control. The control is aliquoted into 1 mL vials and frozen at -20°C. Stable for 12 months.

EQUIPMENT:

- Bench centrifuge
- Gas liquid Chromatograph Schimadzu G-2010 (GLC-FID) system equipped with Autosampler AOC-20i and data interpretation system CLASS-VP 7.2.1

QC MATERIAL:

Internal: A pooled serum control from normal patient sera is used as an internal control. A control aliquot is thawed and run with each analysis.

External: None available.

PROCEDURE:

A. **Total Fatty Acids in Plasma/Serum:** Plasma/Serum requires no special preparation.
B. **Red Cells** are prepared as follows:

- Centrifuge blood at 3000 rpm for 10 minutes.
- Remove plasma into labelled tube and store at 2-4 °C.
- Wash red cells twice by resuspending in 0.9% saline, centrifuging at 3000 rpm, and aspirating off the supernatant.
- Store red cells at-20 °C until assay.

Procedure for preparation of methyl ethers of fatty acids

- To 10ml extraction tube add: 350 µl of plasma/serum/ Quality Control and 1.5mL red cells extract.
- Add 3.8mL of methanol/chloroform mixture to extraction tubes.
- Vortex all tubes for six minutes.
- Add 0.8mL of 0.1 M KCl solution.
- Multi Vortex all tubes for three minutes.
- Centrifuge at 3000rpm for ten minutes.
- Discard aqueous for upper layer by aspiration.
- Place a silane treated glass wool in the bottom of glass Pasteur pipette and fill with sodium sulphate.
- Pass organic layer through sodium sulphate and collect eluate in 2mL vials.
- Evaporate solvent to dryness in heating block (temp <45°c) with **nitrogen.**
- Reconstitute dry residue with 130 µLof Meth-Prep II methylation agent.
- Close vials and leave to stay at room temperature overnight.
- Inject 0.4 µl for **A** and 0.8 µl for **B** of esterification mixture into GC-FID.

- Chromatographic Conditions:

Detector Temperature 300 C
Injector Temperature 250 C
Column Oven Temperature Program

Rate	Temp	Time
0.00	120.00 C	1.50 min
40.00	160.00 C	5.00 min
1.00	185.00 C	0.00 min
2.10	200.00 C	0.00 min
50.00	240.00 C	3.00 min

Injector

Temperature:	250.00°C
Injection Mode:	Splitless
Sampling Time:	0.5min
Flow Control Mode:	Head pressure
Pressure:	17.0psi
Column Flow	1.4 mL/min
Linear Velocity:	37.0 cm/s
Purge Flow:	1.0mL/min
Split Ratio:	-1.0

CALCULATION:

- Export results from Shimadzu integration system CLASS-VP-7.3.1 into Excel macro, rcefa or plasma efa.
- Calculate results expressing them as a relative percentage of the total fatty acids to one decimal place using the Excel macro located in the Chemserve computer, rcefa (for red cell EFA) or plasmaefa (for plasma EFA).

ACCEPTANCE CRITERIA:

- Enter QC results into QC On Call program.
- For results to be accepted, the quality control results must be within the range specified in the QC program for the current batch.

UNITS:

- Units: %
- Report To: One decimal place.
- Reporting Limit: 0.1%

REFERENCE RANGE:

Reference ranges for Essential Fatty Acids in plasma, serum, red cells and platelets are derived from the current literature.

Serum/Plasma	Myristic acid (C14:0)	0.3-1.9 %
Serum/Plasma	Palmitic acid (C16:0)	17.1-19.62 %
Serum/Plasma	Palmitoleic acid (C16:1n7)	1.03-2.07 %
Serum/Plasma	Stearic acid (C18:0)	6.4-7.6 %
Serum/Plasma	Vaccenic acid (C18:1 n7)	1.39-1.83 %
Serum/Plasma	Oleic acid (C18:1 n9)	15.57-20.65%
Serum/Plasma	Linoleic acid (C18:2n6)	31.39-39.09%
Serum/Plasma	alpha-Linolenic acid (C18:3n3)	0.33-0.61 %
Serum/Plasma	gamma-Linolenic acid (C18:3n6)	0.32-0.66 %
Serum/Plasma	Arachidic acid (C20:0)	0.08-0.26 %
Serum/Plasma	Gondoicacid(C20:1n9)	0.08-0.18 %
Serum/Plasma	Eicosadienoic acid (C20:2n6)	0.15-0.29 %
Serum/Plasma	Eicosadienoic acid (C20:3n9)	
Serum/Plasma	Eicosatrienoic acid (C20:3n6)	1.35-2.05 %
Serum/Plasma	Arachidonic acid (C20:4n6)	6.92-10.04 %
Serum/Plasma	Eicosapentanoic acid (C20:5n3)	0.32-0.92 %
Serum/Plasma	Docosapentanoic acid (C22:5n3)	0.44-0.7 %
Serum/Plasma	Docosahexanoic acid (C22:6n3)	1.38-3.2 %
Serum/Plasma	Total Saturated	24.17-29.93%
Serum/Plasma	Total Monounsaturated	18.07-24.81%
Serum/Plasma	Total n3	2.47-5.43 %
Serum/Plasma	Total n6	40.13-52.13%

Serum/Plasma	Ratio n3/n6	0.06-0.1%
Serum/Plasma	Ratio C26:0/C22:0	0.00-0.02%
Serum/Plasma	Ratio C24:0/C22:0 Ratio C20:4n6/C20:5n3 - ideal 1.5 good 3	0.48-0.89%
Red Blood Cells	Myristic acid (C14:0)	0.0-0.7%
Red Blood Cells	Palmitic acid (C16:0)	9.3-21.7%
Red Blood Cells	Palmitoleic acid (C16:1 n7)	0.0-0.4%
Red Blood Cells	Stearic acid (C18:0)	9.3-13.7%
Red Blood Cells	Vaccenic acid (C 18:1 n7)	7.5-15.5%
Red Blood Cells	Oleic acid (C18:1n9)	0.0-1.6%
Red Blood Cells	Linoleic acid (C18:2n6)	5.0-12.4%
Red Blood Cells	alpha-Linolenic acid (C18:3n3)	0.0-0.1%
Red Blood Cells	gamma-Linolenic acid (C18:3n6)	0.1-0.2%%
Red Blood Cells	Arachidic acid (C20:0)	0.1-0.5%
Red Blood Cells	Gondoicacid(C20:1n9)	0.0-0.4%
Red Blood Cells	Eicosadienoic acid (C20:2n6)	0.0-0.2%
Red Blood Cells	Eicosatrienoic acid (C20:3n6)	0.9-2.8%
Red Blood Cells	Arachidonic acid (C20:4n6)	6.2-13.7%
Red Blood Cells	Eicosapentanoic acid (C20:5n3)	0.6-2.8%
Red Blood Cells	Behenic acid (C22:0)	0.1-1.2%
Red Blood Cells	Docosapentanoic acid (C22:5n3)	1.9-4.7%%
Red Blood Cells	Docosahexanoic acid (C22:6n3)	2.5-7.5%
Red Blood Cells	Total Saturated	19.3-39.4%
Red Blood Cells	Total Monounsaturated	7.5-17.9%
Red Blood Cells	Total n3	4.5-13.4%
Red Blood Cells	Total n6	12.1-29.2%
Red Blood Cells	Ratio n3/n6 Ratio C20:4n6/C20:5n3 ideal 1.5 good 3	0.37-0.46%

REFERENCES

1. Edward N. Siquel and al, Clinical Chemistry 33/10 1869-1873; 1987.
2. David F. Horrobin, Clinical Uses Of Essential Fatty Acids; 1982 Essential Fatty Acids: Review pp. 3-25
3. Matreya, Inc. Pufa-2 Standard Chromatogram Catalogue No. 1081.
4. Jeanne D. Joseph, Robert G. Ackman, Journal Of Aoac International. Vol. 75, No. 3,488-506; 1992.
5. Charles B. Berde. and al. Clinical Chemistry 26/8.1173-1177; 1980.
6. Pierre Scribe and Al, Analytical Chemistry 60,928-31; 1988.
7. Supelco, Inc.-Bulletin 721 "Estrification and acylation".
8. Ruud B.H. Schutgens, and al, Clinical Chemistry 39/8.1632-1637; 1993.
9. Jing Ma Aaron and al, Clinical Chemistry 39/12.2527-2528; 1993.
10. A.Kumps.Y.Mardens.Clinical Chemistry 30/2.342-343; 1984.

Attachment:

- Sample chromatogram
- Shimadzu instrument conditions.